Biology of the Baltic Sea

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Biology of the Baltic Sea

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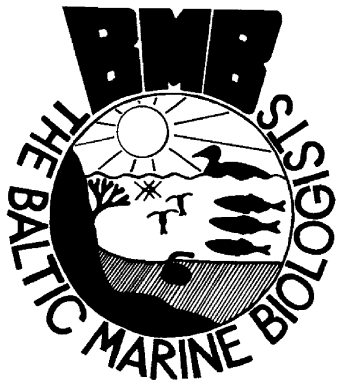

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TABLE OF CONTENTS

Preface ix–x

PLENARY

New perspectives through brackish water ecology
R.E. Ulanowicz 3–12

ECOPHYSIOLOGY

Physiological and proteomic responses in *Mytilus edulis* exposed to PCBs and PAHs extracted from Baltic Sea sediments
B. Olsson, B.P. Bradley, M. Gilek, O. Reimer, J.L. Shepard, M. Tedengren 15–27

Modelling of pristine depth limits for macrophyte growth in the southern Baltic Sea
A. Domin, H. Schubert, J.C. Krause, U. Schiewer 29–39

Active carbon uptake in *Laminaria digitata* and *L. saccharina* (Phaeophyta) is driven by a proton pump in the plasma membrane
M. Klenell, P. Snoeijs, M. Pedersén 41–53

TROPHIC INTERACTIONS

Growth and C:N:P ratios in copepods grazing on N- or Si-limited phytoplankton blooms
L. Van Nieuwerburgh, I. Wänstrand, P. Snoeijs 57–72

Effects of grazing and excretion by pelagic mysids (*Mysis* spp.) on the size structure and biomass of the phytoplankton community
E. Lindén, H. Kuosa 73–78

Food and habitat choice of the isopod *Idotea baltica* in the northeastern Baltic Sea
H. Orav-Kotta, J. Kotta 79–85

Blue mussels (*Mytilus edulis*) in the diet of roach (*Rutilus rutilus*) in outer archipelago areas of the western Gulf of Finland, Baltic Sea
A. Lappalainen, M. Westerbom, S. Vesala 87–92

The effects of turbidity and light intensity on the consumption of mysids by juvenile perch (*Perca fluviatilis* L.)
M. Granqvist, J. Mattila 93–101

POPULATION STRUCTURE AND DYNAMICS

The growth of pikeperch (*Sander lucioperca* L.) and perch (*Perca fluviatilis* L.) under different water temperature and salinity conditions in the Curonian Lagoon and Lithuanian coastal waters of the Baltic Sea
L. Ložys 105–113

Effects of temperature on egg and larval survival of cod (*Gadus morhua*) and sprat (*Sprattus sprattus*) in the Baltic Sea – implications for stock development
A. Nissling 115–123

COMMUNITY STRUCTURE AND DYNAMICS

Influence of dissolved organic matter from terrestrial origin on the changes of dinoflagellate species composition in the Gulf of Riga, Baltic Sea
I. Purina, M. Balode, C. Béchemin, T. Poder, C. Vérité, S. Maestrini 127–137

Temporal and spatial patterns of crustacean zooplankton dynamics in a transitional lagoon ecosystem
Z.R. Gasiūnaitė, A. Razinkovas 139–149

Classification and description of phytobenthic communities in the waters of the West-Estonian Archipelago Sea
G. Martin, K. Torn 151–162

Phytobenthos and macrozoobenthos of the Slupsk Bank stony reefs, Baltic Sea
E. Andrulewicz, L. Kruk-Dowgiallo, A. Osowiecki 163–170

Climatic and hydrological controls over the zoobenthos in a southern Baltic coastal lagoon
T. Radziejewska, M. Chabior 171–181

MODELLING

Effects of land-rise on the development of a coastal ecosystem of the Baltic Sea and its implications for the long-term fate of ^{14}C discharges
L. Kumblad, U. Kautsky 185–196

INVASIVE SPECIES

Distribution, population structure and salinity tolerance of the invasive amphipod *Gmelinoides fasciatus* (Stebbing) in the Neva Estuary (Gulf of Finland, Baltic Sea)
N.A. Berezina, V.E. Panov 199–206

Establishment of the zebra mussel, *Dreissena polymorpha* (Pallas), in the Neva Estuary (Gulf of Finland, Baltic Sea): distribution, population structure and possible impact on local unionid bivalves
M.I. Orlova, V.E. Panov 207–217

The round goby (*Neogobius melanostomus*) in the Gulf of Gdańsk – a species introducton into the Baltic Sea
M.R. Sapota 219–224

EUTROPHICATION

Baltic Sea eutrophication: area-specific ecological consequences
C. Rönnberg, E. Bonsdorff 227–241

How does eutrophication affect different life stages of *Fucus vesiculosus* in the Baltic Sea? – a conceptual model
R. Berger, L. Bergström, E. Granéli, L. Kautsky 243–248

Can different vegetative states in shallow coastal bays of the Baltic Sea be linked to internal nutrient levels and external nutrient load?
S. Dahlgren, L. Kautsky 249–258

Factors controlling long-term changes of the eutrophicated ecosystem of Pärnu Bay, Gulf of Riga
J. Kotta, M. Simm, I. Kotta, I. Kanošina, K. Kallaste, T. Raid 259–268

SOCIETY INFORMATION

Baltic Marine Biologists (BMB)
271

Hydrobiologia **514:** ix–x, 2004.
H. Kautsky & P. Snoeijs (eds), Biology of the Baltic Sea.

Preface

This volume presents some of the results of the 17th Symposium of the Baltic Marine Biologists (BMB), which was held during the Baltic Sea Science Congress in Stockholm, Sweden in November 2001 (BSSC 2001). Scientific research in the Baltic Sea area becomes increasingly interdisciplinary, integrated and co-operational and the BSSC 2001 provided a broad international forum for biologists, geologists and oceanographers to meet and present the latest scientific progress, review previous research and predict needs for future research efforts.

The Baltic Sea (water area ca. 415 000 km^2) is not large compared to the world's oceans, but it is a very important water body for the ca. 85 million people living around it in Denmark, Estonia, Finland, Germany, Latvia, Lithuania, Poland, Russia and Sweden. For them, a good understanding of the system is of paramount interest for nature conservation and a sustainable use of the Baltic Sea resources. The Baltic Sea is also one of the best investigated water bodies in the world and serves as a model for other brackish-water areas world-wide. The Baltic Sea is special, geologically by its young age, geographically by its large drainage area, physically by its low temperature and low salinity, and biologically by its low species diversity and low functional diversity. It is a large experiment of nature where evolution takes place along the long and stable gradients and Baltic ecotypes develop. It is also an area which is especially threatened by human impacts because of its large drainage area and the delicate ecological balance of the system. These aspects of the Baltic Sea, and more, were discussed during the congress, which included 19 interdisciplinary plenary lectures and four symposia with 113 oral presentations, 178 poster presentations, 6 large workshops and many smaller ad-hoc meetings. The five major themes of the congress were: global perspectives, future research perspectives, climatic change, eutrophication and contaminants. One of the highlights of the congress was the participation of His Majesty King Carl XVI Gustaf of Sweden in the plenary 'Royal Seminar' on 26 November. Altogether, 430 scientists participated in the congress, including ca. 250 biologists.

The congress was hosted by the Stockholm Marine Research Centre (SMF) at Stockholm University and the organizing committee consisted of Björn Ganning, Lena Kautsky, Kerstin Ernqvist, Ulrika Brenner and Annika Tidlund from SMF, Pauli Snoeijs and Hans Kautsky from BMB, Mats Ohlson from the Conferences of Baltic Oceanographers (CBO) and Thomas Andrén representing the Baltic geologists. The organizational assistance of many PhD-students from Stockholm and Uppsala Universities during the congress is gratefully acknowledged. Financial support for the congress, including this publication, was received from three main contributors: the Stockholm Marine Research Centre, the Konung Carl XVI Gustafs Stiftelse för Forskning och Utbildning and the Marianne & Marcus Wallenbergs Fond, as well as from the Swedish branch of the World Wide Fund for Nature (WWF), the Swedish Research Council (VR), the Swedish National Board of Fisheries (Fiskeriverket), the Swedish Environmental Protection Agency (SNV), the Swedish Meteorological and Hydrological Institute (SMHI), the Henrik Granholms Stiftelse and the Umeå Marine Research Centre.

This proceedings volume starts with a paper on theoretical ecology, featuring interesting viewpoints on brackish-water ecosystems, by Professor Ulanowicz (University of Maryland, USA), who was one of the invited speakers in the congress. The volume further spans from ecophysiology of Baltic Sea plants and animals to population ecology, community ecology (including trophic interactions), systems ecology and human impacts on the ecosystem by eutrophication and introductions of non-indigenous species. Examples of the latter group of species are the Baikalian amphipod *Gmelinoides fasciatus*, the Ponto-Caspian mussel *Dreissenia polymorpha* and the Ponto-Caspian fish *Neogobius melanostomus*. New data on distributions and ecological characteristics of these invasive species are presented in this book. Several papers present important ecological information on the two

major key-stone species of the Baltic Sea, the bladder wrack (*Fucus vesiculosus*) and the blue mussel (*Mytilus edulis*). These two marine species penetrate far into the brackish Baltic Sea and constitute the only large perennial macroalga and the major filtrating animal in most of the Baltic Sea. Some of the studies on trophic interactions presented here are of major interest in ecosystem functioning, especially in the light of the changing ecosystem with eutrophication, climatic change, contaminants and invasive species. Is the ecosystem of the Baltic Sea changing fundamentally? This is one of the crucial questions for the near future and one of the major challenges for Baltic Sea scientists right now. It is our hope that this proceedings volume, with in all 24 carefully selected peer-reviewed papers from the 17th BMB Symposium, will serve as a good basis for reference within the given framework.

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New perspectives through brackish water ecology

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Key words: ecology, brackish water, Baltic Sea, Chesapeake Bay

Abstract

According to conventional wisdom, the brackish water ecology of the Baltic, like all ecology, is a secondary science. That is, the phenomena it considers can be decomposed into series of more elementary events acting under a sequence of laws that culminates either in the netherworld of quantum physics or in the realm of the cosmological. Ecology, however, is not a derivative science; it is fundamental in its own right. The Baltic ecosystem, for example, is a complex system of many-components. Using combinatorics one may argue that most of the whole-system configurations which ecologists encounter comprise unique and original events that elude treatment via the conventional Baconian approach. Chaos does not reign, however, because there exist among the populations of the ecosystem self-reinforcing mutualistic loops that exert a form of selection upon their constituent members quite different from the 'natural selection' of evolutionary theory. This feedback gives rise to what Karl Popper described as 'propensities' that serve in contingent systems in lieu of conventional forces to maintain the coherence of the ecosystem. The ensuing autonomous 'ecodynamics' can be quantified using information theory, resulting in measures that can be used to compare the status of the Baltic ecosystem with those of similar bodies of water, such as Chesapeake Bay.

Introduction

The reports of the Plenary Session of the BSSC Congress 2001 that appear elsewhere in this volume represent some of the finest marine science being done on the Baltic Sea – and some of the best anywhere, for that matter. There are many adjectives one could use to describe such work – 'pioneering', 'insightful', 'extremely useful', and 'provocative' are a few that might come to mind. I wish to suggest yet another that some readers might find strange or misplaced, namely 'fundamental!'

'Brackish water science, fundamental?', one might ask in astonishment, 'How could such a thing possibly be?' After all, fundamental science is commonly reckoned to be what physicists do when they break apart atomic particles, or what cosmologists do when they estimate the age of the most distant matter. No one considers dragging a net through the water to sample cod to be a contribution to fundamental science. Why not? Because throughout the entire academic realm it is *implicitly* assumed that the events one directly experiences are always derivative. That is, the goal of science usually is to interpret the events one encounters directly in terms of smaller, more short-lived phenomena that may occur beyond one's immediate senses. Or, if such 'reductionism' is not one's cup of tea, one might imagine a universe where the unchanging and universal physical constants combine somehow to 'select' the material forms that are observed.

Such is the conventional wisdom on how one should view the natural world, and I choose to call these seldom-discussed assumptions, 'Genesis at the extremes'. In other words, one is to believe that original causes (if that term has any meaning) can arise only at the edges of existence – either among a completely random netherworld of atomic and molecular events or at the almost unimaginably distant reaches of time and space. Once they originate, however, it is

assumed that their effects propagate to affect everyday experience according to concatenations of strict and inviolable laws. According to this view, the worlds of ecology, and of brackish water ecosystems, are like puppets that dance to tunes played by distant musicians. The ecologist's humble function in this scheme of science seems to be merely to elaborate the lawful mechanical threads that connect what is seen, like the number of cod in the Baltic, to phenomena one causal step removed, such as the rate of fishing or the amounts of phosphorus put into the watershed.

Indeed, it is difficult to imagine any other scenario that makes as much overall sense. Nevertheless, I would like to suggest at least one other possibility – an alternative that places ecology at the very center of what is happening; one that accepts that unique and spontaneous events can arise within ecosystems and affect other events up *and down* the scales of space and time (Ulanowicz, 1999). To elaborate this distinctly ecological paradigm, I will make reference to several ideas that for years now have been discussed among the sub-discipline called systems ecology, and I will try to give them more formal expression using propositions that have been expressed by two recent philosophers of science, Popper (1982, 1990) and Rosen (1985, 1991).

Truly unique events?

I begin by questioning the assumption of 'causal closure'. What this postulate means is that nothing truly spontaneous or original can happen at intermediate scales. Everything one sees in everyday life is the consequence of lawful mechanisms that involve material objects. True, as common wisdom teaches, some systems look quite random and disorganized, and the only mathematics available to describe them is probability theory. But the common belief is that things only *seem* random, and if one were able to gain sufficient and precise knowledge of what is happening among the system elements, it would become apparent that all are behaving in lawful, predictable ways. Whence, according to the assumption of causal closure, randomness is a pure illusion and not a real state of affairs.

Although he was not the first to suggest it, Popper (1982) claimed that the universe is truly open. New and spontaneous events actually happen. Physicists who follow the Copenhagen School of quantum theory seem willing to accept this view, but only insofar as it pertains to events at very small scales. They note that when the randomness appearing at microscopic scales is viewed *en mass*, it usually averages out, and the aggregate follows a wholly predictable course. But Popper would not agree that randomness can occur *only* at the quantum level. No, he holds that truly unique and original events arise at macroscopic scales as well – for example at the scale of the reader's immediate senses.

How could this possibly happen? It is crucial here to point out that classical science always deals with simple systems – systems comprised of only one or a very few elementary material forms, where each form is represented by identical tokens that either act independently of each other or at most interact minimally. Under such conditions, there exists reasonable likelihood that the same configurations will occur again and again. Such repetition is essential to the empirical style of science proposed by Francis Bacon. With simple systems one consistently sees lawful reproducibility.

It is gradually becoming apparent, however, that not all systems are simple. Take the Baltic ecosystem, for example. Probably every Baltic ecologist, if supplied with a decent handbook, would have no difficulty going into the field or afloat on the Baltic and identifying, say, 100 distinct species of plants and animals. But it is not necessary to be even that complicated. About 40 of the most important types that inhabit the Baltic will more than suffice. Now, because this list will be comprised mostly of multicellular plants and animals, one could employ tools like biometrics or genetics to distinguish individual organisms within the groups. Again, to be quite conservative, it will be assumed that only 10 distinct individuals populate each category. In response to the question, 'How many configurations of these 400 individual organisms could be considered as separate events?', the branch of mathematics called combinatorics reveals that the answer is roughly 400! ($400 \times 399 \times 398 \times \ldots \times 3 \times 2 \times 1$), or about 10^{870} ($10 \times 10 \times 10 \ldots$ 870 times).

Now 100^{870} is an *immense* number of events. But the word 'immense' is used here not only according to its conventional meaning. The total is also immense in the sense defined by the late physicist/biologist Elsasser (1981) as transcending the bounds of the physical universe. Elsasser estimated according to the cosmology of his day that the known universe contained approximately 10^{85} elementary particles, and that 10^{25} nanoseconds of time (billionths of a second) have transpired since the Big Bang. He concluded that a maximum of approximately 100^{110} simple events

could have elapsed over the lifetime of the known world. Any combination that would require significantly more than this number of events before it reappeared, quite simply is not going to do so. It must be considered a unique, one-time event. Now, one may quibble about the correct magnitude of Elsasser's threshold or about the actual number of combinations possible in a given system, but his conclusion remains inevitable. As soon as one begins to consider even moderately complex systems, one immediately encounters events that will never be repeated over the lifetime of our physical universe – whatever that may be. Furthermore, after one's eyes have been opened to the possibility of unique events, seeing them *everywhere* becomes unavoidable!

A world of propensities

What does this ubiquity of one-time events imply for the advancement of science? Does the existence of truly random events at larger scales mean that scientists should forget about trying to comprehend biological dynamics? Is it impossible to make any sense out of how the Baltic ecosystem is behaving? Of course not! – and Popper never intended any such pessimistic conclusions. Those familiar with Popper's dialogs with the early postmodernists are aware that he never envisioned a world of only chaos and absurdity. No, Popper clearly recognized that the world is organized, but not in a seamless way. He maintained that there always remain holes in its causal fabric.

In order to approach such a world in a rational manner and achieve what he called 'an evolutionary theory of knowledge', Popper (1990) recommended two actions: (1) It is necessary to reconsider how causality is viewed. Simple mechanism and material object may be insufficient to the task of science. (2) The simple unconditional probabilities, currently in use to quantify systems that are wholly disorganized, are inadequate to describe behaviors in an organized, yet contingent universe. It is therefore necessary to place greater emphasis upon so-called conditional probabilities that are appropriate to a world of occasional chance events, but which for the most part describe systems that appear to take on habits (Hoffmeyer, 1993), or exhibit what Popper called '*propensities*'.

The living world that Popper describes is not one governed entirely by rigid and unyielding laws, which he maintained are appropriate only in perfect isolation. Rather, organization arises out of the propensities for forms and relations to persist over time, but not without occasional lapses, which he called *interferences*. Now, rigid laws yield unvarying results: If A, then B; if A, then B; if A, then B; … etc., *ad infinitum*. If I were to suspend a ball in my hand and release it, it would certainly fall to the earth. By contrast, if I say that given A, there is a *propensity* for B to happen, it means: If A, then B; if A then B; … if A, then B; if A, then C(!); if A then B; if A then D(!); etc. A cod feeds, it ingests a sprat; a cod feeds, it ingests a sprat; a cod feeds, it ingests a sprat; …; a cod feeds, it ingests a squid(!) As just mentioned, such propensities are related to what are called *conditional* probabilities. Furthermore, Popper emphasized that propensities never occur in isolation, but always within a context (which may include other propensities). When the context changes, propensities can alter accordingly, just as the cod would likely change its dietary habits if a significantly new collection of prey were suddenly available to it.

A world of selection

A world of propensities is certainly a rich and interesting place, but the question immediately arises, 'Why habits at all?' (Indeed, it is passing strange that hardly anyone ever asks the question, "Why laws?') Exactly what is it about ecosystems and other living systems that might foster the formation of habits? Why isn't all biological behavior completely opportunistic and random? The answer, as the reader may have already guessed, is *'selection'*. Of course, that word is immediately associated with the natural selection of evolutionary theory, and to be sure, natural selection does play some role in ecosystem dynamics. But Darwin was careful to portray natural selection as something acting independently of the organism or system being studied – in pure form, something like the physical conditions of temperature, oxygen, or available space. Furthermore, it always acts in a negative way, i.e. it selects *against* unfit participants. Thus, natural selection remains external to the system to eliminate unfit specimens and, in tautological and directionless fashion, passively leaves those capable of surviving, simply to survive.

At the risk of appearing radical, I wish to suggest that a more immediate form of selection is at work as ecosystems develop – a form of selection quite unlike the conventional 'natural selection' in that it acts from *within* the ecosystem and establishes a

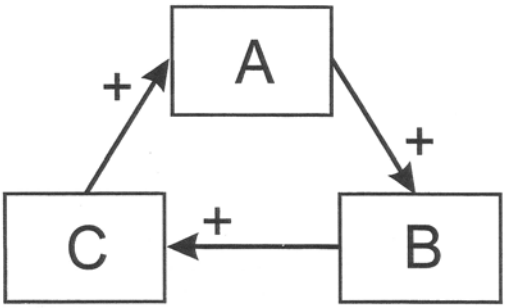

Figure 1. Schematic of a hypothetical 3-component autocatalytic cycle.

preferred *direction* for subsequent changes. Such selection is generated by generalized mutualism, or what in chemistry has been called 'autocatalysis' (Ulanowicz, 1997). An example of generalized mutualism could be the 3-element cycle of relationships shown in Fig. 1.

In this scheme one assumes that the processes associated with A have the *propensity* to augment those processes connected with B. I wish to emphasize the use of the word 'propensity' to mean that the response of B to A is neither obligatory nor exclusive. That is, A and B are not tightly and mechanically linked. Rather, whenever the processes associated with A increase in magnitude, most (but not all) of the time, those connected with B also will increase. The action of B tends to accelerate those of C in similar fashion, and C has the same effect upon A.

A particular instance of this configuration could be something like the cycle of material transfers in the Baltic ecosystem that occurs from (A) 'deposit feeders' to (B) 'carnivorous fish' and then to (C) 'sediment carbon' and back to (A) again. 'Deposit feeders' (A) include bottom-dwelling mussels, crabs and snails that feed on particles of detritus that fall to the bottom. Carnivorous fish (B), such as cod and herring, use these deposit feeders as food. The fish in their turn generate particulate waste (C) that falls to the bottom and provides food for the deposit feeders.

My favorite ecological example of autocatalysis exists outside the Baltic and is the freshwater community associated with the aquatic macrophyte, *Utricularia* (Ulanowicz, 1995). All members of the genus *Utricularia* are carnivorous plants. Scattered along its feather-like stems and leaves are small bladders, called utricles. Each utricle has a few hair-like triggers at its terminal end, which, when touched by a feeding zooplankter opens the end of the bladder and the animal is sucked into the utricle by a negative osmotic pressure that the plant had maintained inside the bladder. In the field *Utricularia* plants (A) always support a film of algal growth known as periphyton (B). This periphyton in turn serves as food for any number of species of small zooplankton (C). The three element catalytic cycle is completed when the *Utricularia* captures and absorbs many of the zooplankton.

Now, it follows almost by definition that autocatalysis is explicitly *growth-enhancing*. It increases the level of activity by all members of the loop. More important to my argument is that autocatalysis is capable of exerting *selection pressure* upon its ever-changing constituents. To see this, one assumes that some small change can occur spontaneously in B. If that change either makes B more sensitive to A or a more effective catalyst of C, then the change will receive increased stimulus (reward) from A. Conversely, if the change in B either makes it less sensitive to the effects of A or a weaker catalyst of C, then that alteration will likely diminish its own support from A. I note that such selection works on the processes or mechanisms as well as on the elements themselves. Hence, any effort to simulate development in terms of a fixed set of mechanisms is doomed ultimately to fail.

It should be noted in particular that any change in B is likely to involve a change in the amounts of material and energy that flow into the system to sustain B. Whence, a corollary of selection pressure is seen to be the tendency to reward and support changes that bring ever more resources into B. Because this reward process pertains as well to all the other members of the feedback loop, any autocatalytic cycle thereby becomes the center of a *centripetal* vortex, pulling as many resources as possible into itself. In such centripetal action the autocatalytic configuration is not simply reacting passively to its environment, but rather is an active agency that changes its surroundings.

It follows from centripetality that, whenever two or more autocatalyic loops draw from the same pool of resources, autocatalysis will *foster competition*. In particular, whenever two loops partially overlap, the outcome could be the exclusion of one of the loops. In Fig. 2b, for example, element D is assumed to appear spontaneously in conjunction with A and C. If D is more sensitive to A and/or a better catalyst of C, then there is the likelihood that the ensuing dynamics will so *favor* D over B, that B will fade into the background or disappear altogether (Fig. 2c). That is, autocatalysis selects in a *positive* way those ele-

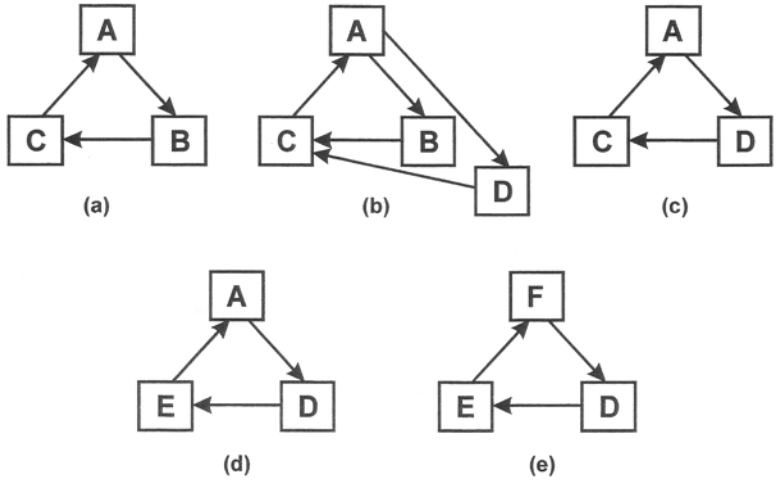

Figure 2. (a) Original autocatalyic configuration. (b) Competition between component B and a new component D, which is either more sensitive to catalysis by A or a better catalyst of C. (c) B is replaced by D, and (d & e) the loop section A-B-C by that of F-D-E.

ments and mechanisms that favor an increase in its own action.

Of course, if B can be replaced by D, there remains no reason why C cannot be replaced by E (Fig. 2d) or A by F, so that the cycle A,B,C could eventually transform into D,E,F (Fig. 2e). One concludes that the characteristic lifetime of the autocatalytic form usually exceeds that of most of its constituents. This is not as strange as it may first seem. With the exception of neurons, virtually none of the cells that composed an individual's body seven years ago remain in place today. Very few of the atoms currently in that body were parts of it eighteen months ago. Yet if the mother of the subject were to see him/her for the first time in ten years, she would immediately recognize her child.

A depiction of the effects of autocatalysis upon relationships in the system might look something like the cartoon shown in Fig. 3. On the top is shown a hypothetical, immature 4-component network before autocatalysis has emerged. Below is depicted the same system after autocatalysis has matured. The magnitudes of the flows are represented by the thicknesses of the arrows.

Taken together, selection pressure, centripetality and a longer characteristic lifetime all point to the existence of a degree of *autonomy* of the larger structure from its constituents. Again, attempts at reducing the workings of the system to the properties of its composite elements will prove futile over the long run. Ecology is not simply a derivative of physics, chemistry or even of evolutionary theory.

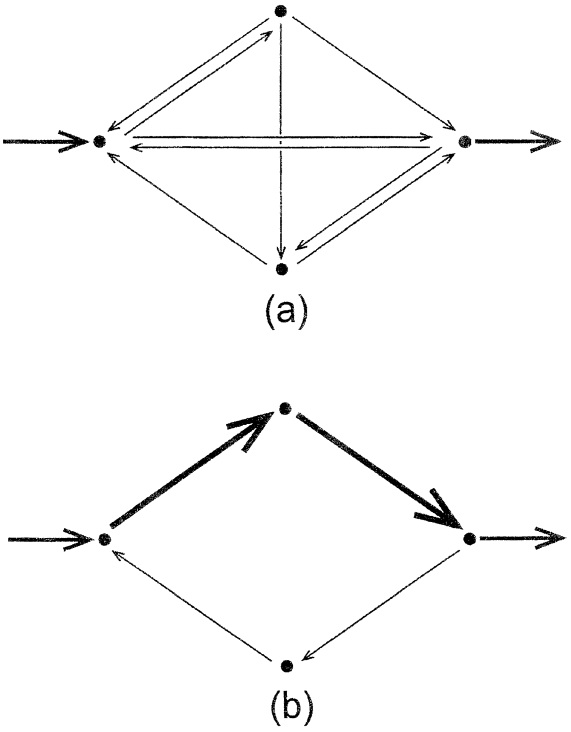

Figure 3. Schematic representation of the major effects that autocatalysis exerts upon a system. (a) Original system configuration with numerous equiponderant interactions. (b) Same system after autocatalysis has pruned some interactions, strengthened others, and increased the overall level of system activity (indicated by the thickening of the arrows).

Causality reconsidered

By accepting the possibility of some degree of autonomous behavior in ecosystems, one is actually adopting a new perspective on causality, as Popper had advocated. It is significant that the selection generated by autocatalysis is exerted by a larger, longer-lived configuration upon its smaller, more transient constituents. Because autocatalysis is always the result of a formal configuration of processes, it becomes appropriate to follow the lead of Rosen (1985) and speak of this 'top-down' influence as an example of *formal cause* sensu Aristotle.

The possibility of top-down formal causality gives a very different color to ecology than one finds in evolutionary theory. The noted philosopher, Dennett (1995), for example, describes the progressive complexity of biological entities as analogous to 'cranes built upon cranes', whereby new features are hoisted on to the top of a tower of cranes where they then become the top crane that lifts the next stage into place. Dennett cautions, however, against considering any causality from above that does not have its foundations among smaller-scale mechanical agencies. Such prohibited agencies he calls a 'skyhook'.

I acknowledge Dennett's warning against skyhooks. I fear, however, that his mechanical crane metaphor leads one astray from the true face of nature, which in the case of living systems needs to be more organic in form. This point was driven home to me in one of the very few 'Eureka!' events that I have ever experienced (Ulanowicz, 2001). I was working distractedly in my garden, pondering why I thought Dennett's analogy was inappropriate, when my eye was drawn to a muscadine grapevine that has grown on the corner of my garden fence for the last twenty-five or so years. In the initial years after I had planted it, the lead vine had climbed the fence to become a central trunk that fed a lattice-work of grape-bearing vines (Fig. 4a). Eventually, the lateral vines had let *down* adventitious roots that met the ground somewhat less than a meter from the trunk (Fig. 4b). Then in the last few years, the main trunk had died and rotted away completely, so that the framework of vines was being sustained by the new roots, which themselves had grown to considerable thickness (Fig. 4c).

There are no skyhooks here! The system always remains in contact with a foundation of bottom-up causalities that remain a necessary part of the narrative. It is the later structures, however, that *create* connections which eventually replace and *displace*

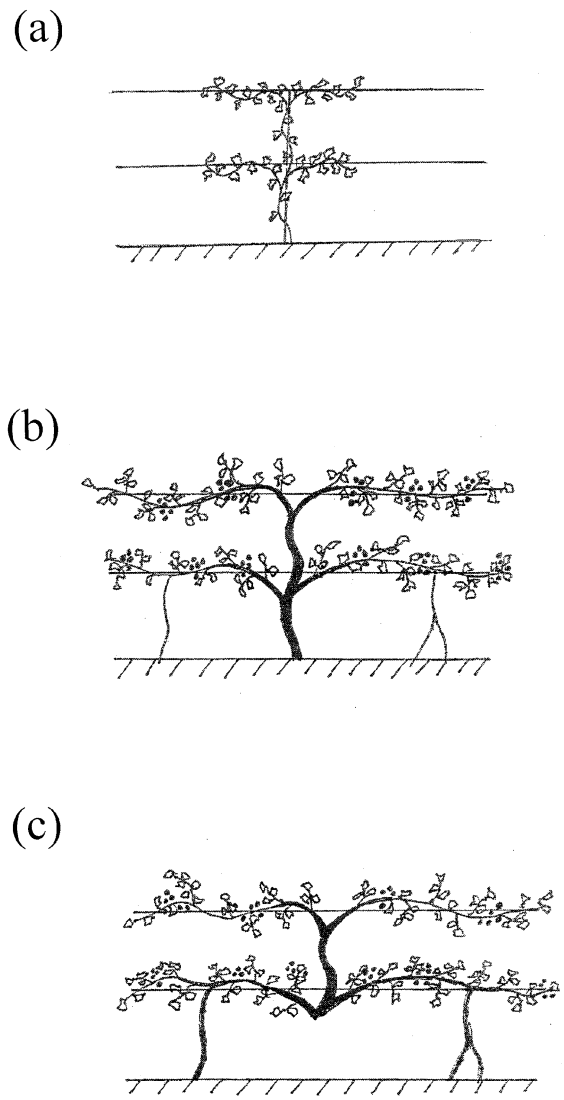

Figure 4. (a) Young muscadine grapevine with central stem and branches. (b) Grapevine several years later, having developed adventitious roots to the sides of the main trunk. (c) Same grapevine two decades later. Original trunk has rotted away, but vines are sustained by adventitious root system.

their earlier counterparts. Top-down causality, totally alien to mechanistic-reductionistic evolutionary discourse, nevertheless fits the developmental situation perfectly. Development is like a muscadine grapevine. As strange as that analogy might seem at first, it describes development (and I would suggest also evolution) more fully than Dennett's mechanical construct.

Finally, to return to the directionality engendered by autocatalysis, it is an example of what physicists call *symmetry-breaking*. It is a strict departure from the symmetrical, non-directional world of physics. I be-

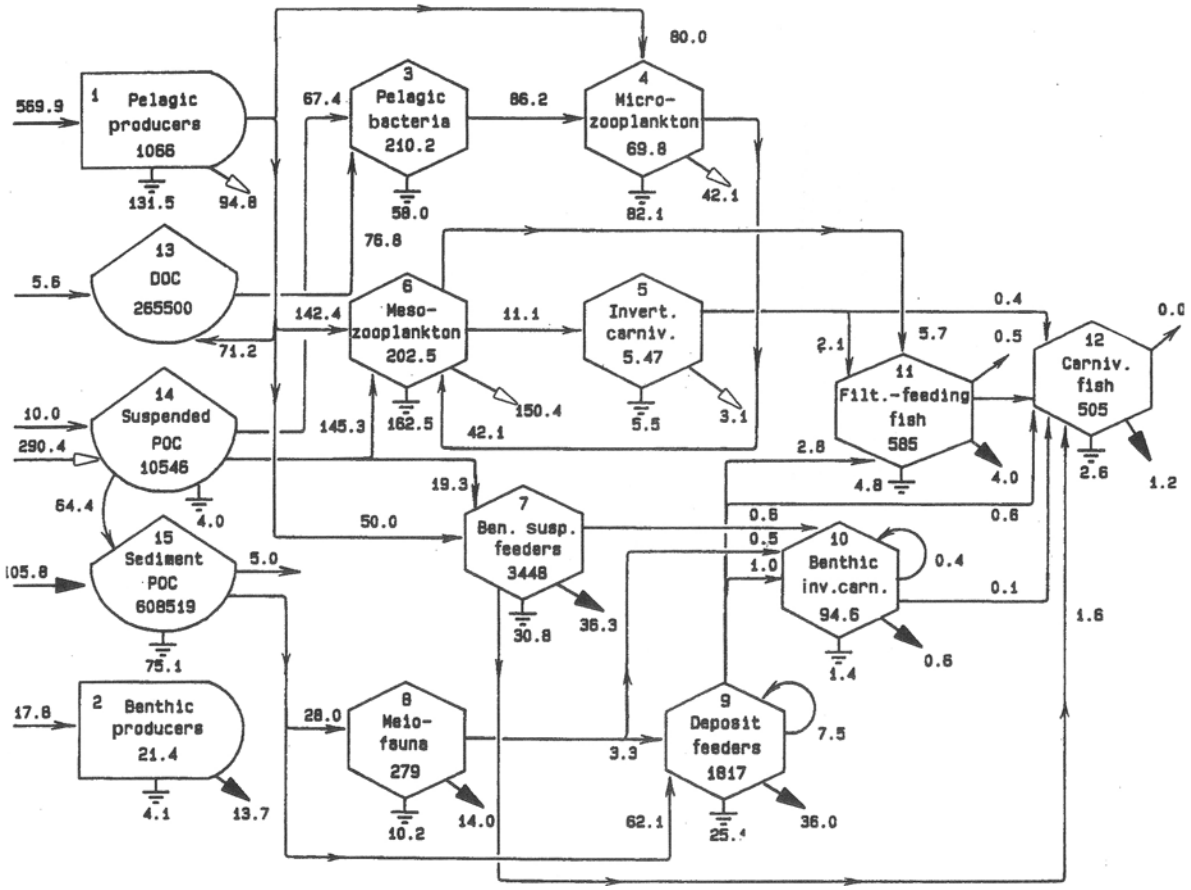

Figure 5. Diagram of the annually-averaged exchanges of carbon among the 14 major trophic aggregations of the ecosystem of the Baltic Sea. Units of biomass (inside boxes) are mg C m^{-2}; units of flows, mg C m^{-2} d^{-1}. Return flows to suspended Particulate Organic Carbon (14) are represented by open arrows; those to sediment POC (15), by filled arrows. Ground symbols represent dissipative respiration.

lieve Rosen would also suggest that such directionality resembles Aristotle's final cause, only it is far more elementary in nature. I caution that one should not confuse this very simplistic directionality with full-blown teleology. It is not necessary, for example, that there exist a pre-ordained endpoint towards which the system strives. The direction of the system at any one instant is defined by its state at that time, and the state changes as the system develops. For these reasons, I have chosen the term '*telos*' to denote this weaker form of directionality and to distinguish it from the rarer and more complex behavior known as teleology.

Ecodynamics

The stage has now been set to compare ecodynamics, as just described, with the conventional view of how systems operate and evolve. The common wisdom teaches that any truly original event can arise only in the microscopic realm of sub-atomic, or at most, molecular phenomena. Once an original change has occurred there, focus abruptly shifts in almost schizoid fashion, to the lawful world of organisms and other larger structures. But the common assumption is that nothing truly new can happen in this macroscopic world. It is the realm of regular and reproducible phenomena. A truly chance event at this level would upset the entire framework of precise, deterministic laws. According to these laws, those entities that are less able to act in coherence with the overall physical structure of their 'environment' are eliminated. As the adapted survivors reproduce, focus returns momentarily to the stochastic netherworld of molecules, where variety can emerge once more. It is by the accumulation of such microscopic variety that the living world is able to adapt to changes in the overall physical environment.

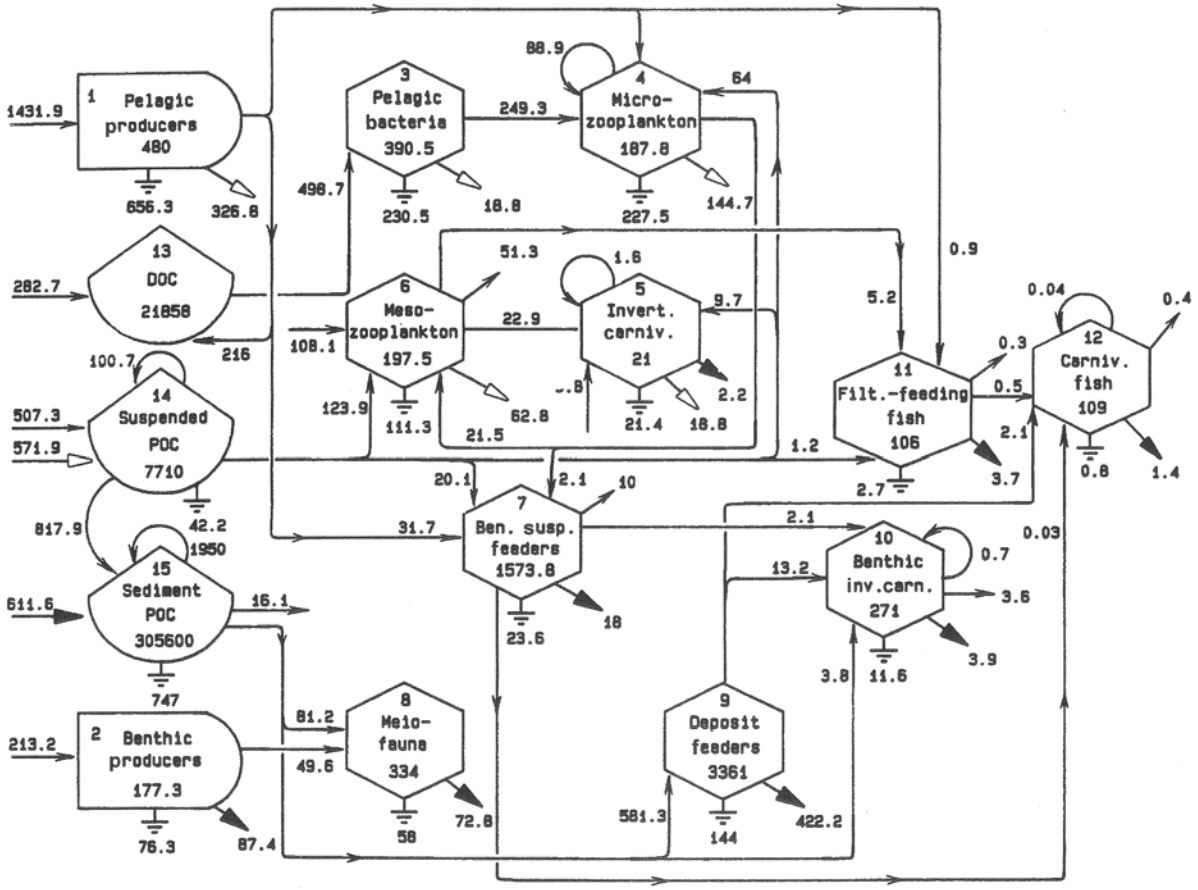

Figure 6. Diagram corresponding to Fig. 5 for Chesapeake Bay ecosystem.

The key difference in the ecodynamical scenario is that unique, original events may occur at *any* scale. They are appearing all the time, everywhere. Unlike in the accepted view of dynamics, most such genetic events do not threaten the established organization and remain of little or no consequence. Such neutral variety is allowed simply to accumulate. Occasionally, configurations arise that are deleterious to the autocatalytic feedbacks imbedded among the system structures, and these forms eventually fade from the scene, because they diminish their own feedback supports. On other rare occasions new configurations appear that resonate with the existing feedback dynamics, and these structures amplify to the extent that they displace some of the neutral variety.

This last point reveals a certain antagonism in the ecodynamic scenario between two opposing tendencies: The first is the propensity for autocatalysis to displace members of the system that do not contribute to its own activities. The second tendency is simply for neutral forms to accumulate. In the first instance, selection favors more highly constrained, efficient and organized networks of interactions, or ones with what I have called more 'ascendency'. In contrast, the collection of marginal, inefficient and disorganized processes gives rise to what I call the system's 'overhead'. In a purely mechanical world, the efficiencies associated with ascendency would eventually crowd out all the remaining overhead, resulting in a maximally efficient ecosystem configuration, or what Herbert Spencer called the 'survival of the fittest'.

The real world, however, doesn't seem to behave in such an extreme way. A maximally efficient system is also maximally constrained. It is 'brittle', in the words of Holling (1986) and unable to adapt to any change in its environment. Although inefficiencies and incoherencies tend to degrade the system performance (ascendency), they nevertheless also provide degrees of freedom that become absolutely essential to the survival of a system that has been subjected to a novel dis-

turbance. When subjected to perturbation, the existing overhead offers the system potential repertoires that it can adopt to survive under the new circumstances. Without sufficient overhead, a system is unable to *create* effective responses to the challenges presented by its environment.

One concludes that natural ecosystems must always consist of some combination of disorganized freedom to adapt (overhead) as distinct from its organized functioning (ascendency). The actual ratio of flexibility to constraint will be influenced by the nature of the physical environment surrounding the system. In benign, almost predictable environments, such as that of the tropical rain forest, one expects to find proportionately more ascendency; whereas in rigorous environments, such as the boreal steppe, one anticipates a greater proportion of overhead.

Parsing constraint from flexibility

Thus far, I have engaged only in 'armchair theorizing', but science must be quantitative and operational. It is beyond the scope of this paper to explain in detail how ascendency and overhead can be measured (Ulanowicz & Norden, 1990). It is possible, however, to discover a hint of how it is done by referring again to Fig. 3, which illustrates the effects of autocatalysis on a system. The reader will recall that the top figure depicts an immature system with many ambiguities and flexibilities, i.e. one rich in overhead. By contrast, the bottom one shows a system that is very tightly constrained; one that is strictly organized (high ascendency) and inflexible.

That is to say, the structures of networks that depict how populations in the ecosystem are related to one another provide clues as to the relative amounts of organization vs. flexibility in the system. In the early 1940s the American ecologist Lindeman (1942) began a long tradition in ecosystems science by creating diagrams that were constructed in response to the questions, 'Who eats whom?' and 'By how much?' Fig. 5, for example, depicts the sources and average magnitudes of carbon that are ingested by each major group of consumers in the Baltic Sea ecosystem, as estimated by Dr Fredrik Wulff and his associates in the Department of Systems Ecology at Stockholm University (Ulanowicz & Wulff, 1991).

Applying a branch of mathematics called 'information theory', it becomes possible to quantify the variety of the processes that appear in this diagram. More importantly, however, the same mathematics can be used to separate the fraction of this variety that is expressed as constrained organization from how much remains as flexibility. That is, one can supply numbers to the word equation:

$$\text{Variety} \;=\; \text{Organization} + \text{Flexibility}.$$

In the case of the Baltic diagram just shown, 56% of the variety of processes appears as organization, while 44% remains as flexibility.

Baltic ecologists, of course, would be most interested in comparing the organizational status of their ecosystem with those of similar habitats elsewhere. In Fig. 6 is depicted another network that colleagues of mine estimated in similar manner for the Chesapeake estuary in the USA (ibid). When the organizational status of the Chesapeake is evaluated, it exhibits less organization (50%) and more disorganization (flexibility) than the Baltic. This evidence (along with others) led Prof. Wulff and myself to the unexpected conclusion that the Baltic is less disturbed than the saltier Chesapeake. This judgement contradicts the common wisdom that fresher water ecosystems are more vulnerable to nutrient additions than saltier ones. It would appear, then, that the 'health' of the Chesapeake ecosystem, as indicated by these measurements, has been more degraded in recent years than has been the case with the Baltic.

The ability to assess the overall health and integrity of an ecosystem is, of course, of great practical and political importance. But to Baltic ecologists I would like to suggest that ascendency theory conveys a message of even greater psychological importance. Over the entire history of ecology, it has been tacitly assumed that ecologists are second-class members of the scientific community. Whatever they may uncover, as exciting as it might be, they remain faced with the discouraging belief that their discovery must be analyzed further in terms of more fundamental events that lie the domains of colleagues in the fundamental sciences, like physics, chemistry, or evolutionary theory.

But with the new ecodynamics that I have just outlined, and with the help of tools such as ascendency theory, scientists are slowly coming to realize that they can observe directly some primary causes as they occur among the ecosystems they study (and, incidentally, among economic and social systems as well). Some of the phenomena depicted in these networks did not arise out of quantum or molecular physics. In fact, to reverse the tables somewhat, much of what persists in the forms of specific biomolecules (e.g.

DNA sequences) or mechanisms have been selected by relational configurations that exist at the level of the whole ecosystem. That is, genes and mechanisms come and go as transients in a larger and more persistent *ecological* dynamic.

In closing, I wish to encourage brackish water ecologists to take heart! For they are not laboring in the shadows of some higher echelon of scientists who have a monopoly on basic research. When next they drag their net through the waters, when again they drop their corer into the muddy bottom, as they continue to labor at the microscope, counting the densities of copepods or algae, I would like to remind them that they are dealing with matters that arise, at least in part, out of causes that appear only over the length and the breadth and the depth of the Baltic Sea – not in some lifeless microscopic realm or in some cold distant reach of space. Baltic ecologists are engaging in *fundamental* research!

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Physiological and proteomic responses in *Mytilus edulis* exposed to PCBs and PAHs extracted from Baltic Sea sediments

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Abstract

Stress responses in blue mussels (*Mytilus edulis. L.*) exposed to organic pollutants were measured using several physiological measures and as changes in protein expression. Blue mussels from the Baltic Sea were exposed for 6 days in a flow-through system to two fractions of extracted Baltic sediments (containing primarily PAHs or PCBs) from one industrially impacted site and one off-shore site. Exposure to Aroclor1248 (a commercial PCB mixture) was included as a reference treatment. Physiological response was measured as changes in respiration, excretion, clearance rates and scope for growth. Of the physiological responses, only clearance rate and scope for growth in the Aroclor and impacted site PCB treatments differed significantly ($p < 0.05$) from control organisms, perhaps due to a large variation among individuals. Seven proteins were observed, presumed to be from stress protein families (hsp60, hsp70 and hsp90) on one-dimensional electrophoresis gels. All protein levels, except three proteins, 62, 73 and 90 kDa, in response to PCB exposure from the industrial site, were significantly higher ($p < 0.05$) in treated than in control organisms, suggesting the use of stress-inducible proteins as diagnostics in risk assessment. A wider sample of proteins was observed using two-dimensional gel electrophoresis. The presence or absence of protein spots compared to control organisms was used as an indication of stress. Between 23 and 76 proteins or spots were present and 15 to 23 absent compared to controls, and the results supported the physiological and one-dimensional gel results, suggesting that the mussels were indeed suffering from stress. The methods used here represent stress monitoring at two different levels of biological organization; the cellular- and the level of individual organisms. In this experiment the protein response showed less variation among individuals compared to the physiological parameters. The protein response, however, still suffers from the lack of interpretation into commonly used monitoring terms, which emphasizes the need for more knowledge of whether the response is a momentary reflection of exposure or an early warning of higher order effects.

Introduction

Proteins are of fundamental importance for all organisms, as they are used for maintenance, growth and reproduction. The formation and proper exposure of the active sites that give proteins biological functions are dependent on the tertiary structures of the proteins. Those structures have chemical bonds that are vulnerable to stressful conditions like heat or toxic chemicals, which implies that stress can negatively affect biological functions (Hochachka & Somero, 1984; Somero, 1995). Breakage of the chemical bonds eventually leads to partly unfolded proteins that expose regions that are satisfied in the native state, which leads to the binding to other such unsatisfied regions, and the result is formation of aggregates. The aggregated proteins can not maintain the native functions of the proteins, which severely affects the organism and ultimately causes mortality. The evolutionary solution to this cellular problem is a class of proteins called

chaperones (Ellis, 1987). Their functions associated with aggregated proteins are today well established (Ellis, 1999). Chaperones under stressful conditions are involved in the protection of organisms on the cellular level. It has been proposed that the chaperones would be suitable as a sensitive environmental monitoring tool. In this perspective the chaperones together with several other stress-induced proteins are commonly termed stress proteins (Bradley, 1993; Sanders, 1993; Wiens et al., 1998).

Environmentally stressful conditions cause changes in the levels of many proteins, not just those termed 'stress proteins' and proteins of the proteome are either suppressed or induced as a response to stressed situations (Bradley et al., 1994). Proteomics has already been applied in the clinical and biomedical fields where alterations in the proteome are measured and correlated with the occurrence of disease and the action of drugs (Blackstock & Weir, 1999). Protein alterations can identify some change in the environmental state of an organism but without understanding the protein function or correlation to higher order effects the organismal significance of these changes remains a mystery, although some research has attempted to correlate the induction to higher levels of fitness or environmentally relevant conditions (Roberts et al., 1997; Werner et al., 1998; Hofmann, 1999). Stress protein responses have been correlated to both physiology and ecology (Sanders & Martin, 1991; Krebs & Loeschke, 1994; Chapple et al., 1998; Hightower et al., 1999; Hofmann, 1999). The stressed state on this level would thus have the potential to be an indicator of stress well before other stress effects are observed, and as ecologists studying toxicological problems we are especially interested in this use of the protein response.

In our earlier work with *Mytilus edulis* we have found that physiological parameters such as respiration-, excretion- and clearance rates are correlated to stress protein induction. However, the results have also shown that stress protein induction is more complex than might be expected. We have found that the stress protein response in *M. edulis* is altered by heat treatment prior to cadmium exposure. There was increased induction of hsp70 compared to mussels exposed to cadmium only (Tedengren et al., 1999a). We have also found that the result depends on which stress protein is studied. When investigating a set of six proteins we found that all but one reached a peak level followed by a decline in mussels exposed to lowered salinity, copper, Aroclor 1248 and combinations (Olsson et al., submitted). Comparing survival rates of *M. edulis* from the brackish Baltic Sea with mussels from the marine North Sea after exposure to the same kind of stress, revealed significant differences correlating well with induction of hsp 70, suggesting that background conditions are important when discussing intraspecific differences in induction (Tedengren et al., 1999b).

The mixture of anthropogenic stress factors in the Baltic Sea, including a variety of compounds from industry and agriculture as well as diffuse impact from traffic, surface water run-off and municipal waste (Leppäkoski & Bonsdorff, 1989), combined with the natural low salinity in the Baltic Sea demonstrates how disturbance commonly occurs as a complex phenomenon in the environment. This in turn leads to symptoms that are not specific enough to determine the origin of the stress, and emphasizes the need for a sensitive tool that can distinguish the effects of stress caused by human activities from natural stress early in exposure.

The focus in the present study was to investigate two ways of using proteins as monitoring tools to detect reactions to chemicals introduced in the ecosystem by man. In order to do this we compared the results of physiological measurements, 1-dimensional analysis of heat shock proteins and a wider (proteome – using 2-D gels) analysis of mussels exposed to mixtures of PCBs and PAHs extracted from sediments from two sites, one reference site and one more polluted, in the Baltic Sea. This comparison also provided the opportunity to compare the results of the cellular response with the response at the organism level.

Material and methods

Sampling and treatment

Blue mussels (1.8–2.3 cm in length) were collected from about 3 m depth at a natural habitat near the Askö Laboratory in the northern Baltic Sea proper (58° 49′ N, 19° 39′ E). Ambient salinity and temperature at the collection site were 6.3 psu (practical salinity units) and 7 °C respectively. The mussels were freed from epibionts and kept in a 30-l tank with continuous exchange of filtered seawater of ambient salinity and temperature for 7 d and the mussels were continuously fed with cultured algae (*Chlamydomonas* sp.) during the acclimatization phase.

The experiment was performed with two types of chlorinated organic compounds, mainly PCBs and

PAHs. The different treatments were exposure to field sampled PCBs and PAHs and to the earlier commonly used PCB mixture Aroclor1248. The latter compound is a chemical product specified in terms of congeners and concentrations within certain limits, to fulfill requirements from the commercial users. This compound was used as the treatment reference to the field sampled PCB. In contrast to the reference, the PCB- and PAH-extracts were supposed to have a more heterogeneous composition.

Surface sediments (0–2 cm) from an offshore reference site in the central Bothnian Sea (SR5) and from a site outside a pulp and paper mill (Iggesund) were collected in November 1992 using a gravity core sampler of Kajak type. Sediment samples of 8 g dry weight (dwt) were Soxhlet extracted with toluene and HPLC fractionated as described by Engwall et al. (1997). The experiments described in this paper were performed on two fractions from each sediment: (1) Diaromatic compounds (e.g. PCBs, PCDD/Fs and PCNs) and (2) Polyaromatic compounds (e.g. PAHs).The diaromatic and polyaromatic fractions were analysed for their contents of PCBs and PAHs respectively as described by Broman et al. (1994). The concentrations of the 14 analyzed PCB congeners, including all of the ICES 7, at the Iggesund site were found to be 4183 pg g dwt^{-1} and at the reference site 6141 pg g dwt^{-1} – a surprisingly high value. The concentrations of the analyzed PAHs at the Iggesund site were found to be 1041 ng g dwt^{-1} and at the reference site 336 ng g dwt^{-1}.

The experimental regimes were made of a mix of 3 l of filtered seawater, 150 ml of the algae culture (resulting in 2×10^5 cells l^{-1}) and the toxicants. The available food is thus in the lower range of food ratio, strongly affecting the kinetics of PCBs according to Björk & Gilek (1997). The control mixtures were prepared in the same manner excluding the addition of chemicals. This resulted in the following experimental regimes ($n = 4$): control, 1.5 ng PCB L^{-1} for the industrial site (Iggesund), 2 ng PCB L^{-1} for the reference site (SR5), 347 ng PAH L^{-1} for the industrial site (Iggesund), 112 ng PAH L^{-1} for the reference site (SR5) and a reference mixture prepared from the Aroclor 1248 and set to 100 ng L^{-1}.

Mussels were then exposed for 6 days at ambient temperature and salinity to the five experimental regimes as well as the control regime. During this time all mussels attached and seemed to filter, judging by the open shell valves and visible gills. There was no mortality during the experiment. At the end of the experiment it was recognized that the treated mussels were not as strongly attached to the substrate as the control mussels (the cause was a visually identified reduced byssus thread production). Four replicate 0.5-l beakers with 5 mussels in each were used for each treatment. The experiment was carried out using a flow through system in a randomized block organization.

The mixtures were distributed to the beakers from 3-l treatment tanks with a peristaltic pump set at rate of 0.5 l per 24 h. Each of the five 3-l experimental mixtures as well as the control water was evenly distributed into the 4 exposure beakers within each treatment.

All chemical analyses of PCB and PAH fractions followed standard procedures (see e.g. Broman et al., 1991).

Physiological measurements

After the 6 day exposure period, physiological rates were measured. Respiration rates were measured as the rate of decline in oxygen partial pressure for 2 h in the experimental beakers ($n = 4$) using a polarographic oxygen electrode (Microprocessor Oximeter, OXI 196). Ammonia excretion levels were estimated according to standerd methods. Immediately after this the clearance rates were measured using a particle counter (Elzone 280 PC). Water samples were taken at the start and after 30 min, three replicate counts were made on each sample. Clearance rate was calculated according to the formula: Clearance rate $(\mathrm{lg}^{-1}\mathrm{h}^{-1}) = (\ln C_g - \ln C_0) - (\ln C_1 - \ln C_0) \times V \times B^{-1} \times T^{-1}$, where C_0 is the particle concentration at time T_0, C_1 is the concentration at time T_1, C_g is the particle concentration in chambers without mussels at time T_1, V is the volume of suspension, B is the dry tissue weight of the mussels. T is the elapsed time between readings. The absorption efficiency was determined according to the method of Conover (1966). The mussels (with shells) were placed in a $-80\,^{\circ}\mathrm{C}$ freezer immediately after the physiological measurements for later biomass determinations and protein extractions. All rates were transformed by means of appropriate weight exponents, to compensate for minor differences in size between individuals, and the results used to calculate an energy budget for each treatment according to Tedengren et al. (1999a).

One-dimensional protein extraction and determination

Soluble proteins were extracted from 12 whole individual mussels from each treatment by mechanical

homogenizing in ice cold phosphate-buffered saline (PBS) (150 mM NaCl, 10 mM Na_2HPO_4, pH 7.2) with 1 mM phenylmethylsulfonyl fluoride (PMSF). Protease inhibitors were used and samples were centrifuged at 18 000 ***g*** for 20 min at 4 °C, according to the method of Bradley & Ward (1989). Supernatants were collected for the protein analysis. Total protein concentration in each sample was determined based on the method of Bradford (1976). The 12 individual whole mussel homogenates were loaded on to 3 different gels (4 mussels per gel) runned in triplicate.

Protein separation

Proteins were run on 12.5% SDS-PAGE (sodium dodecyl sulphate-polyarylamide gel electrophoresis) gels using the BioRad Protein II xi Cell. An amount of 150 μg of total protein was added to each lane. Samples were mixed 1:4 with denaturing sample buffer and heated at 95 °C for 5 min before being loaded to the lanes. The gels were electrophoresed at room temperature with cold tap water running in the cooling core for approximately 5 h, using discontinuous (Laemmli, 1970) running buffer. Coomassie Brilliant blue staining method was used for the visualization of proteins.

Protein identification

After electrophoresis, gels were transferred to nitrocellulose membranes using electrophoretic transfer system. Blots were blocked with PBS-Tween (0.3%) and incubated for 1 h with monoclonal hsp70 antibody (Sigma) (1:500 in PBS-Tween), rinsed with PBS-Tween and incubated with goat anti-mouse alkaline phosphatase-conjugated antibody (Sigma) (1:1000 in PBS-Tween) for 1 h. The nitrocellulose then was rinsed and incubated in pH 9.5 buffer (0.1 M Tris, 0.1 M NaCl and 5 mM $MgCl_2$) with nitroblue tetrazolium and 5-bromo-4-chloro-3 indolyl phosphate (NBT-BCIP) for colour development.

The other (putative) stress proteins were identified with known molecular weight standards (Myosin 202 000 daltons; β-galactosidase 133 000; Bovine serum albumin 71 000; Carbonic anhydrase 41 800; Soybean trypsin inhibitor 30 600; Lysozyme 17 800; Aprotinin 6900) from BioRad) These proteins are referred to as p58, p62, p65, p82 and p90 with molecular weights of approximately 58, 62, 65, 82 and 90 kDa, respectively. Unknown proteins were determined according to the relationship between the relative mobility and $\log_{10}$ known molecular weight, according to the method of Guttman et al. (1980). By these methods it is not possible to distinguish between inducible and constituitive forms of heat shock proteins, but since we only compare protein concentrations between treatments compared to control, this is of no significance to our conclusions.

Protein quantification

Gels were scanned using Molecular Dynamics Densitometer. Selected bands were quantified, by means of internal standards as above, using volume integration in a region with defined background. The given unit is pixels.

Two-dimensional protein extraction and determination

Proteins from the gill tissue of four individuals from each treatment group were extracted by mechanical homogenization (Oxford GlycoSystems). Tissue samples were homogenized on ice for 3 min in Sample Buffer I (0.3% sodium dodecyl sulfate, 20 mM Dithiothreotol, 28 mM Tris-HCl and 22 mM Tris Base). Samples were then boiled for 5 min (100 °C). Nucleotides were removed by ice incubation for 15 min with Sample Buffer II [24 mM Tris base, 476 mM Tris-HCl, 50 mM $MgCl_2$, 1 mg ml^{-1} DNase I (Sigma), 0.25 mg ml^{-1} RNase A (Sigma)]. Cellular debris was then removed by centrifugation (13 000 ***g*** for 5 min). The protein fraction was removed from the centrifugation supernatant by the addition of 50% Trichloroacetic acid (TCA) for a 15-min ice incubation. The precipitated protein pellet was formed by centrifugation for 2 min (13 000 ***g***). The protein pellet was washed with 95% acetone and resuspended in Sample Buffer Mix (7.92 M Urea, 0.06 SDS, 1.76% 3–10 ampholytes, 120 mM DTT, 3.2% Triton-X 100, 22.4 mM Tris-HCl and 17.6 mM Tris-base). The solution was centrifuged for 5 min (13 000 ***g***) and the protein pellet once again suspended in Sample Buffer Mix. The amount of total soluble protein was determined according to the protocol of Bradford (1976).

Protein separation

The protein fraction was separated using two-dimensional electrophoresis (Biorad Miniprotean II™). Isoelectric focusing (IEF) gels were cast in vertical capillary tubes following the protocol described in the BioRad Mini-Protean II users manual using 4% gel monomer (9.2 mM Urea, 4% acrylamide, 20%

Triton X-100, 1.6% 5/8 ampholytes, 0.4% 3/10 ampholytes and catalysts). 4 individual mussels were sampled from each treatment, then duplicate protein samples of 30 μg total protein were added to the capillary gels and electrophoresed for 3.5 h at 750 V. IEF tube gels were extruded into two milliliters of Sample Equilibration Buffer (0.065 M Tris-HCl (pH 6.8), 2.3% (w/v) SDS, 5% (w/v) b-mercaptoethanol, 10% glycerol and 0.05% bromophenol blue). Samples were then stored at −80 °Cuntil their separation by SDS-PAGE. Each capillary tube gel was placed in the 2-D well with 12% SDS polyacrylamide slab gel with a 4% stacker gel (Biorad). Protein standards (Promega) with a range of 97.4 to 14.4 kDa were loaded in the marker lane. Samples were electrophoresed at 140 V for 1 h.

Protein visualization

Proteins on gels were visualized using a double neutral silver nitrate stain and fixed in the gel (30% ethanol, 10% acetic acid) overnight. The gel was then washed in 30% ethanol and then washed in reverse osmosis deionized water (RO H_2O). Gels were then incubated in 0.1% $AgNO_3$ allowing time for the silver molecules to bind to the protein. The silver was developed with a solution of 2.5% $NaCO_3$ and 0.02% formaldehyde, after a quick water wash to remove any unbound silver. After development, the protein bound silver was reduced by incubation in Farmer's reducing solution (0.0067 M potassium cyanide and 0.116 M sodium sulfate). Farmer's reducing solution was removed with multiple washes using large amounts of RO H_2O. Gels were then incubated a second time with silver nitrate and developed. Gels were incubated in developer solution until marker bands were fully stained with silver and sample protein spots were visible. Washing the gel for 10 min in 1% acetic acid terminated development of the protein bound silver. Gels were then rinsed in RO H_2O and stored in plastic bags. This technique has a detection limit of approximately 2–5 ng of protein per spot (Biorad, Hercules, California).

Protein repression and induction

Duplicate gels for each of the four individual mussels were scanned to TIFF images (EPSON Expression 636 flatbed scanner, 400 dots per inch). Images were imported into the Medical Electrophoresis Analysis Expert System 2.1 (MELANIE) software (Biorad). Protein spots on each gel were detected using six algorithms to correct for background, horizontal and vertical streaking, and over saturation of spots. 'Landmarks' were placed on all the gels distributed in an approximate 'X' shape across the sample area of the image to allow for the alignment of all gels in the horizontal and vertical directions. Protein standards were always chosen as landmarks together with approximately 20 other protein spots that appeared on every image in all treatment groups. Images from each treatment group were aligned with one another and the $x-y$ coordinate of each spot matched by the computer. Combining individual gels, using spots found on at least 75% of the original gel images produced synthetic (composite) gels representing each treatment. Composite gels from each treatment were compared to the control composite as well as to one another to identify changes in pattern due to treatment with each of the five chemical mixtures. All the information from each composite gel and the relationships to other composite gels was compiled using the feature report database (FREDA 1.4) developed at UMBC, U.S.A. (Shepard et al., 2000; Shepard & Bradley, 2000).

Statistics

The homogeneity of variances was tested with the Cochrans test. Data were log-transformed to correct for any deviation from normality and for lack of homogeneity of variances. Overall treatment effect was tested with a 1 factor ANOVA. The five treatments with 20 mussels in each were then followed up with a *post hoc* multiple comparison test (Student–Newman–Keuls).

Results

The field mixtures of PCBs and PAHs differed in concentration and composition between the two sites. The field extracts of PCB where more heterogeneous than the PCB reference Aroclor, as suggested in the methods. The PCB-extract from the polluted site contained less PCB per gram sediment (4183 pg g dwt^{-1}) than the extract from the offshore location (6141 pg g dwt^{-1}) according to the 14 analyzed congeners (on ICES 7 list + 7 more). This can probably be explained with deposition of persistent chlorinated compounds in deep Baltic sediments and the fact that the diaromatic content (PCDD/F & PCN) probably differs (e.g. Håkanson et al., 1988). The field sampled PCBs were also found to contain site-specific concentrations of the 14 congeners with more persist-

Table 1. (a) Analyses of a subset of 14 PCB congeners from the sediment extract (0–2 cm). Note the higher concentration of heavily chlorinated compounds (degree of chlorination increases with congener number) at the off shore site (SR5) compared to the near shore industrial site (Iggesund). The concentrations are given in pg g dwt^{-1} of sediment. (b) Analyses of 15 compounds with four or more benzene rings, from the sediment extract (0–2 cm). The higher concentrations at the industrial site (Iggesund) than at the off shore site (SR5) reflects that PAHs have a narrower distribution pattern relative to source than PCBs. The PAHs are arranged in increasing log K_{ow} order. The concentrations are given in ng g dwt^{-1} of sediment.

(a) Congeners	Iggesund	SR5	(b) PAH	Iggesund	SR5
# 18	251	18	Flu	199	19.5
# 28	282	130	Pyr	124	12.3
# 52	195	127	2-mePyr	19.9	1.5
# 70	224	210	1-mePyr	14.3	1.1
# 90+101	274	549	BghiF	20.5	4.8
# 118+123	150	317	CpcdP	0	2.2
# 110	214	396	BaA	81.6	9.4
# 105	63	98	Chr	132	29.1
# 153	687	1327	BkF	60.4	26.5
# 149	476	914	BeP	84.2	35.4
# 138	687	1045	BaP	91.7	15.2
# 180	680	1010	Per	37.2	22.3
			Ind	81.4	68.4
Total	4183	6141	BghiP	68.6	47.6
			Cor	25.8	40.9
			Total	1041	336

ent heavy chlorinated congeners in the off shore sediment. The sediment from the industrial site contained higher concentrations of less chlorinated congeners, which are more readily degraded (Table 1a).

The sum of the analyzed PAHs in sediment from the industrial site was higher than at the off shore site, 1040 ng PAH g^{-1} compared to 336 ng g^{-1} (Table 1b). In the case of PAH there was no laboratory-specified compound available, thus no PAH-treatment reference was used. The difference in content and composition of organic pollutants between the offshore and the industrial site, is probably explained by overall hydrodynamics and sedimentation patterns, as well as differential degradation rates due to low oxygen conditions in the deep Baltic Sea (e.g. Broman et al., 1991; Wulff et al., 1993). The differentiated content and composition thus probably also affect the toxicity of the sediment extracts (Baumard et al., 1998a,b).

The physiological parameters indicated stress in the treated mussels (Fig. 1). While respiration and ex-

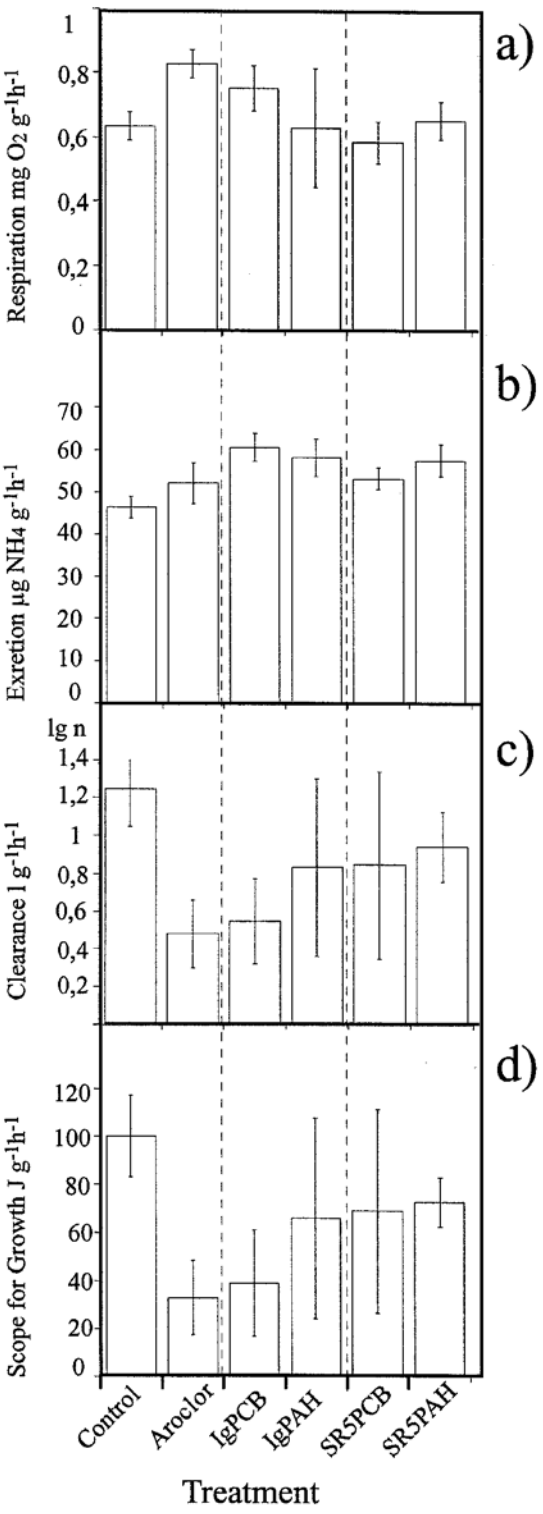

Figure 1. Results from the physiological measurements (mean ± SD).

cretion values were altered slightly there was a steep decline in clearance rates. Aroclor1248 and the PCB fractions from the industrial site lowered the clearance rates by more than 50% ($p < 0.05$). The PAH fraction from the industrial site and also both fractions from the off shore site decreased the clearance rates by about 30% (Fig. 1c). The lowered clearance rates found in these treatments affect the consumption and scope for growth, which altogether is a signal of stress at the organism level (Fig. 1).

Seven proteins (p58, p62, p65, hsp71, hsp73, p82 and p90) were identified and compared (Fig. 2). Each spot represents the mean of triplicate scans of each bar on the lane of 12 mussels. All protein concentrations increased compared to control, most of them at a significant level (Table 2), which clearly indicates a stress reaction. As can be seen in Table 2a–d the PCB fraction from the industrial site raised HPS levels in four of seven cases (p58, p65, p82 and hsp71) compared to control ($p < 0.05$).

All seven proteins seemed to react similarly compared to control though reaching different concentrations (Fig. 2). Consequently, no stressor specific hsp-reaction was observed. This is in accordance with earlier results of Baltic Sea mussels where we have found the same induction trend of those proteins in response to copper, decreased salinity and Aroclor 1248 (Olsson et al., submitted). The reaction of the 65 kDa isomer was somewhat different since this protein was not detectable in any of the analyzed control mussels, but was found in all treated mussels. The induction pattern was however the same as for the other proteins.

Multiple proteins were observed in 2-D patterns of proteins in the Aroclor1248 treated mussels. Aroclor1248 caused a change in protein expression with 23 present protein spots and 16 absent spots, compared to control. The PCB extract from the industrial site caused 76 present protein spots and 23 absent spots compared to control (Table 3a). Of these spots 12 were present and 11 absent in the Aroclor1248 protein pattern (Table 3b). The PCB extract from the offshore site caused 21 present and 15 absent protein spots. Of those 8 present and 10 absent were the same as for the Aroclor treatment. Thus about 50% of the proteins differentially expressed in Aroclor 1248 were detected under field extract exposures. The PAH extract from the industrial site resulted in 64 present spots and the same extract from the off shore site 56 spots. The absent number of protein spots was 16 and 23 respectively. When the two PAH fraction treatments were compared they were found to share 22 present and 7 absent spots (Table 3).

Discussion

We have focused on the use of proteins as tools to detect responses to chemicals introduced in the ecosystem by man. We have used three different ways of measuring stress, one on the organism level: measurements of physiological parameters, and two on the cellular level: response of seven hsps according to 1-D gel analysis and a broader approach now known as proteomics (Wilkins et al., 1996; Williams & Pallini, 1997) with data from 2-D gel analysis.

The methods in the present work each showed that the mussels were suffering from adverse conditions, both due to exposure to PCB (Aroclor1248) and to environmentally occurring mixtures of extracted PCBs and PAHs. The final nominal water concentrations according to the analyzed PCB congeners and polyaromatic compounds were estimated to be 1.4 ng PCB l^{-1} from the industrial site (Iggesund), 2 ng PCB l^{-1} from the reference site, 347 ng PAH l^{-1} from the industrial site (Iggesund) and 112 ng PAH l^{-1} from the reference site. Those values approximate resuspension from a soft bottom, when toxic compounds are bio-available, especially to filter feeders. The nominal Aroclor1248 concentration (100 ng l^{-1}) is high compared to concentrations of organic pollutants in the Baltic Sea. Water column concentrations of PCBs have been found to be in the range 2.4–24 pg l^{-1} for the dissolved fraction and in the range 3.1–70 pg l^{-1} for the particle bound fraction. The corresponding ranges for PAHs are 0.1–8.9 ng l^{-1} and 0.3–2.7 ng l^{-1} (Axelman et al., 1997). It has also been taken into account that the particle concentration in the present work was in such a low range (200 000 algae cells l^{-1}) that reduced filtration activity probably decreased the particle bound uptake of contaminants (Björk & Gilek, 1997). This was also observed as a low filtering activity in the present work. Nevertheless, it has been shown that both water pumping and filtration activities are important factors for PCB uptake and that the proportion of hydrophilic and hydrophobic congeners affect the mechanism and degree of uptake (Björk & Gilek, 1999). We did not analyze the proportion of hydrophilic and hydrophobic components but it is known that the hydrophobic properties of PCB congeners increase with increasing chlorination (Hawker & Connell, 1988). This was also recognized

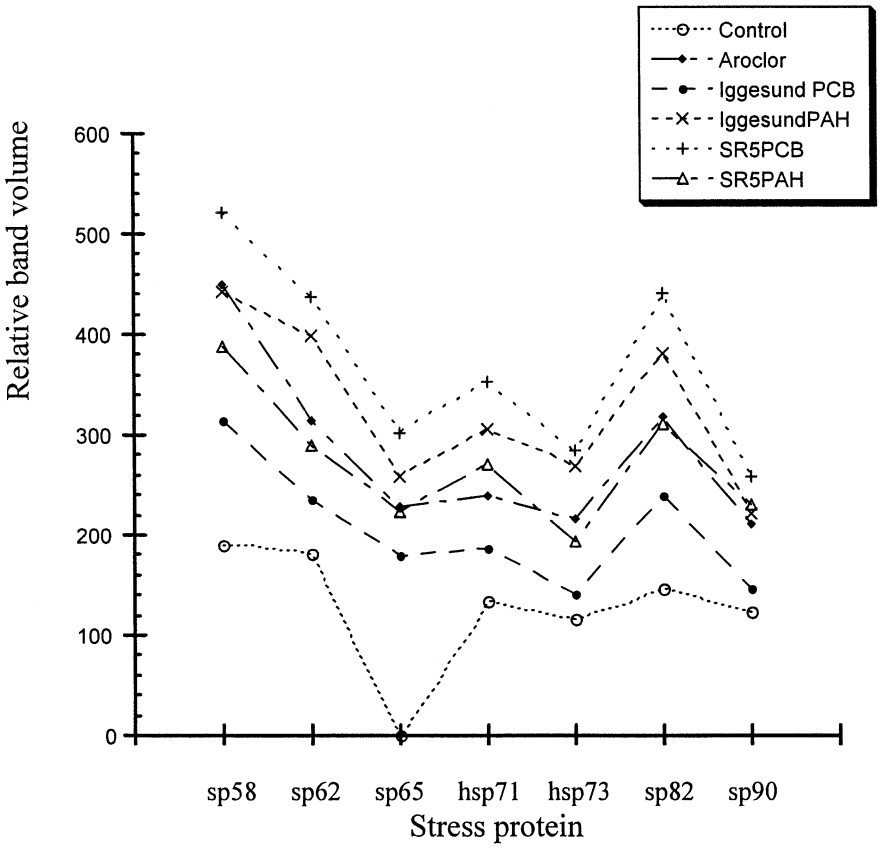

Figure 2. Treatment effects on expression of the seven stress proteins. Each spot represents the mean of triplicate runs of 12 individual samples. The lines are inserted to visualize the similar patterns of induction.

when the selected congeners in both sediment extracts were analyzed, and more heavily chlorinated congeners dominated in particular at the off shore site (Table 1). Polycyclic aromatic compounds like PAHs are in general hydrophobic and thus readily bioaccumulated alternatively bound to sediment particles (Bandh et al., 1996; Baumard et al., 1998a). The metabolism (and biotransformation to metabolites) of PCB and PAH have been shown to differ from invertebrates to vertebrates, causing high levels of bioaccumulation in mussels and severe toxicological effects (Livingstone, 1985; Gilek et al., 1996; Baumard et al., 1998b). A reasonable assumption in the present work is that the low concentration of algae used kept the filtration activity at a moderate or low level, thus under- rather than over-estimating this route of contaminant uptake.

The mussels treated with PCB from the industrial site had very similar physiological reactions to the Aroclor1248 treated mussels, although the concentration of the PCBs was almost 100 × lower than the treatment with Aroclor1248. The explanation for the similar stress reactions may instead be found in the relative composition of congeners and occurrence of other diaromatic compounds (PCDD/F and PCNs) (Engwall et al., 1997).

In earlier studies we have found a very high level of variability in the physiological parameters measured at the organism level, even within the same experiments (Gilek et al., 1992; Tedengren et al., 1999a,b). The high variance found also in this study may be masking real effects in the present work. However, the trend in the physiological response of the treated mussels was clear; the negative effects of the treatments were observed as decreases in clearance rates of up to 50% in comparison to the control organisms (Fig. 1). The lowered clearance rate reduced consumption and ultimately the scope for growth. These results thus indicate, that the experienced stress had effects that will cause higher level implications on the Baltic mussel population and probably result in reduced growth rate and reproductive success or even mortality in the long run.

Table 2. The relative levels of heat shock proteins arranged in increasing order of induction which also affect the arrangement of treatments. Each value represents the mean of triplicate runs of 12 individual samples. The levels are compared pair-wise, to control and to each other. Means joined by underline do not differ significantly according to the Student Newman–Keuls test (*post hoc*).

(a)	Treatment					
	Control	Iggesund	SR5	Iggesund	Aroclor	SR5
Stress protein		PCB	PAH	PAH		PCB
sp58	190.1	314.4	388.7	442.9	449.1	521.3

(b)	Treatment					
	Control	Iggesund	SR5	Aroclor	Iggesund	SR5
Stress protein		PCB	PAH		PAH	PCB
sp62	181.7	235.4	289.0	313.4	397.9	437.1
sp65	0.0	178.5	223.7	228.2	257.7	302.6
hsp73	116.7	140.0	193.6	215.7	269.2	284.9
sp82	146.3	238.9	309.8	317.1	380.6	440.1

(c)	Treatment					
	Control	Iggesund	Aroclor	SR5	Iggesund	SR5
Stress protein		PCB		PAH	PAH	PCB
hsp71	133.7	185.9	239.0	269.9	305.1	353.6

(d)	Treatment					
	Control	Iggesund	Aroclor	Iggesund	SR5	SR5
Stress protein		PCB		PAH	PAH	PCB
sp90	123.6	146.4	210.2	220.4	229.3	258.3

Table 3. (a) Present and absent protein spots compared to control in response to all treatments, according to the comparison of 2-D gels of gill tissue proteins. Duplicate gels were run for each of four individual mussels within treatment. (b) Present and absent protein spots shared between treatments according to the comparison of 2-D gels of gill tissue proteins.

(a)	Treatment	Present protein spots	Absent protein spots
	Aroclor1248	23	16
	Iggesund PCB	76	23
	SR5 PCB	21	15
	Iggesund PAH	64	16
	SR5 PAH	56	23

(b)	Treatment compared	Present protein spots in common	Absent protein spots in common
	Aroclor & Iggesund PCB	12	11
	Aroclor & SR5 PCB	8	10
	Iggesund PAH & SR5 PAh	22	7

The majority of the stress protein levels in all treatment groups were significantly higher than those of the control mussels. The treatment with a PCB fraction from the industrial site did not cause significantly higher levels of 3 of the 7 stress proteins studied (hsp 62, hsp73 and hsp90) (Table 2b, 2d). The reason might

once again be the different composition of the treatment mixtures, in combination with a protein specific response to toxicants. We have observed this differentiated phenomenon in previous work with *M. edulis* from the Baltic Sea, where we found that the increase in protein induction of the six proteins in that study reached a plateau or even started to decrease when the stress reached a certain level (Olsson et al., submitted). Roberts et al. (1997) sampled mussels (*Mytilus californianus*) at different times of the year and found different endogenous levels of different isoforms of hsp70 depending on season. Following this they treated the mussels with elevated temperatures in the laboratory and found that the mussels sampled during summer season with the highest endogenous levels of hsp70, had a dampened induction response compared to the winter sampled mussels. Roberts et al. (1997) argued that the reason for this phenomenon is to be found in the cost of the protein synthesis. The explanation for the decrease in protein synthesis at a certain stress level is thus likely to be found in the context of available energy. This might especially be the case in the Baltic Sea where marine organisms already are under natural osmotic stress (Kautsky 1982; Kautsky et al., 1990). The differential nature of the stress protein response has been described earlier as Sanders et al. (1992) found differentiated induction of hsp 60 and hsp70 and their isoforms in *M. edulis* in response to elevated temperature, and Hightower et al. (1999) described different trigger temperatures for induction of hsp30 and hsp70. Lundebye et al. (1997) determined organotin concentrations in *M. edulis* collected from five natural sites, and compared those concentrations with the hsp60 and hsp70 levels but found no correlation with metal concentration. They concluded that the stress protein response requires further elucidation before it can be used as a biomarker. Schroeder et al. (2000) studied hsp70 induction correlated to DNA damage in environmentally collected North Sea dab *Limanda limanda.* They detected three forms of the protein (hsp70, −73 and −75) and found that they all got induced by the same toxicants but at different levels. Furthermore they found a significant correlation between DNA-damage and stress protein levels. Finally they concluded that it was not possible to correlate the induced stress protein levels to the pollution situation at the sampling sites as there were to many unknown factors in terms of anthropogenic and natural influence. Clayton et al. (2000) investigated the dose-response relationship of the expression of hsp60 and hsp70 in the zebra mussel (*Dreissena polymorpha*)

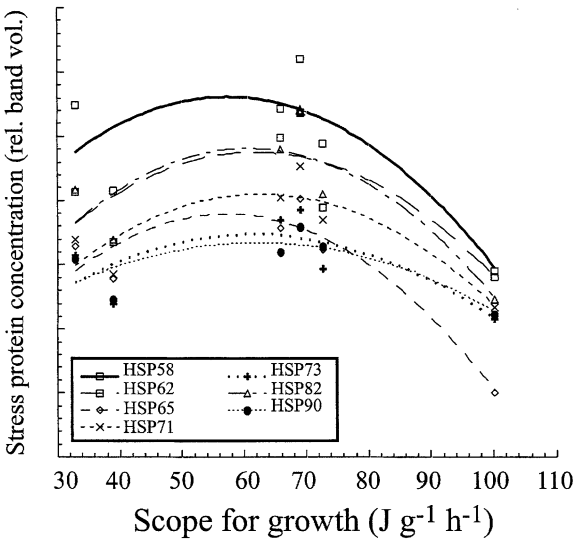

Figure 3. Mean values of all the stress proteins, including control level, analyzed on the 1-D gels plotted against the scope for growth. A binomial curve fit is added for visualization.

following exposure to a range of copper and tributyltin concentrations. They found that hsp60 had a biphasic induction expression pattern and that hsp70 reached a plateau, in response to copper exposure. Under TBT exposure both stress proteins reached a plateau. Those relationships are in agreement with our previous work (Olsson et al., submitted) and also with the present results. Thus these results underline the need for more applied research on stress proteins.

The complexity of the hsp response and difficulty of higher order interpretation was visualized in the present work when the results of the 1-D protein analysis of heat shock proteins were compared with the physiological results (Fig. 3). The physiological results suggested that Aroclor1248 and PCB from the industrial site were the largest stressors (Fig. 1). According to the protein analysis of all seven measured stress proteins the PCB fraction from the reference site was the most severe stress factor with significantly elevated concentration level compared to control and most of the other treatments (Fig. 2, Table 2). We thus found that the level of stress protein compared to control is a reliable tool to identify stressed environments in general, but if the purpose is to estimate how severe different stress factors are compared the each other or to identify a certain stressor the measurement of stress protein levels is not enough (Figs. 2, 3). Werner et al. (1998) exposed amphipods (*Ampelisca abdita)* to sediments from 23 different sampling sites containing heavy metals, PAHs and PCBs. They found correlation

between hsp 36 and hsp64 levels and survival in some cases, but they also found that some sediment lowered the levels of the stress proteins. They argued that hsp60 and hsp70 are good indicators of contamination but not reliable for assessing integrated stress caused by exposure to complex mixtures due to inhibitory, additive and synergistic effects. This is especially true when, as in the present work, it is impossible to rank the treatments, and the dose-response expression patterns of the proteins are different depending both on the stress protein and the contaminant. This is clearly visualized in Fig. 3, where all measured stress proteins are plotted against scope for growth. A certain concentration of stress protein does not correspond to a fixed physiological response (Fig. 3), which makes it difficult to conclude on the dose-response relationship since physiological measures suggest one treatment (Aroclor) to have the greatest impact and protein studies suggests that another treatment (SR5 PCB) is a more potent disturbance (Figs. 2, 3).

We also examined a wider range of possible protein markers of stress, using 2-D gels to determine protein expression changes by comparing presence or absence of protein spots on gels from treated mussels with gels from control mussels. This method indicated that treatment with any of the toxicants caused high levels of changes at the cellular level, indicating a general stress response. In response to the Aroclor1248 treatment 23 proteins were present and 16 absent compared to control, which was a relatively modest change, similar to the response to the PCB fraction from the off shore site with 21 present and 15 absent protein spots. The other three treatments caused present protein spots in the range 56–76 and absent protein spots in the range 16–23 (Table 3a). Different numbers of present and absent protein spots due to different treatments were obvious. In this context the most 'pure' mixture was Aroclor1248, since the other mixtures of PCB and PAH were the results of extraction from field collected sediments, and thus had a more complex composition. The number of protein spots might reflect this.

Treatment with the PCB fraction from the industrial site (Iggesund) resulted in 76 present and 23 absent protein spots compared to the control gels. When the gels also were compared with the Aroclor1248 gels those two treatments were found to share 12 present and 11 absent protein spots compared to control (Table 3b). When the Aroclor1248 gels were compared with the gels of the PCB fraction from the offshore site they were found to share 8 present and 10 absent proteins spots (Table 3b). The proteome response to the two PAH fractions shared 22 present and 7 absent protein spots. This is consistent with our earlier results (Shepard et al., 2000) where we found that differing numbers of specific proteins from each primary toxicant (Aroclor1248, Copper and reduced salinity) was persisting and detectable in a mixture of all the toxicants. As both identity and function of the detected protein spots are unknown at present it is impossible to correlate them to the higher order effects found in the physiological measurements. A reasonable conclusion is, however, that the extensive reaction in terms of absent and present proteins spots compared to control affects energy turnover negatively and thereby most likely reduces Darwinian fitness (sensu Koehn & Bayne, 1989).

Conclusions

All methods used were able to indicate the stressed state of the mussels, irrespective of which toxicant was used. The results of the analysis of heat shock proteins were most consistent from individual to individual. The results of the physiological analyses were highly variable making significant effects difficult to establish at sub-lethal stress levels. However the protein responses were clear and sensitive. The analysis of total protein changes showed a high level of cellular activity for all treatments in comparison to control mussels and was consistent with the results from the heat shock protein analysis.

We found that the level of stress protein compared to control is a reliable tool if the goal is to identify stressful environments in general. If the purpose on the other hand is to identify or compare the severity of a certain stressor, estimation of stress protein levels is not enough, as the differentiated stress response of the isoforms in the present study did not correlate the hsp reaction with any specific stress factor.

The results of the proteomic approach with the 2-D gels with highly differentiated numbers of present and absent protein spots shows the feasibility of this method in distinguishing between different stressors in the laboratory and in field collected sediment samples. The specific protein spot pattern detected as a response to the Aroclor1248 and the PCB-fractions exposure together with earlier results implies that a reference database might be useful in detecting specific stressors in the field.

The dose-response expression patterns of the proteins seem to depend both on the type of stress protein

and the contaminant. These changes need to be linked to higher order effects and the functional significance of the protein changes need to be determined for them to be useful as environmental monitoring tools. In order to achieve this, the natural fluctuations of heat shock proteins and the proteome need to be known so that natural and anthropogenic protein responses can be distinguished. To show the accuracy of the method it is necessary to perform long term experiments where altered protein levels are linked to life history characteristics such as spawning frequency and survival.

Finally, these methods may be complementary. Physiological measures are well accepted and understood, while the protein changes provide more sensitive indicators of general and specific toxicity.

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Modelling of pristine depth limits for macrophyte growth in the southern Baltic Sea

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Abstract

The knowledge of plant and animal distributions within surface water ecosystems is a prerequisite to develop an ecological classification system based on the guidelines of the Water Framework Directive of the European Union (EU-WFD). We developed a system of typology for macrophytes of the inner coastal waters of the German Baltic Sea based on given physical and chemical descriptors of the EU-WFD, and so far known ecophysiological requirements of the plants. Analysis of these requirements led to a minimum matrix of 14 factor combinations for a sufficient ecological characterisation of the communities (Bluemel et al., 2002). Here, we report on a model to describe the pristine habitats based on specific physical and chemical properties and ecophysiological potentials of macrophytes. In order to evaluate the most likely depth limits for macrophyte distribution we calculated annual depth-dependent light intensities for our reference lagoons. Knowledge of minimum light requirements for growth of typical species enabled us to compute maximum depth distribution through the year. Comparison of computed limits for growth were found to be in accordance with historical records. Therefore, we suggest that anthropogenic eutrophication and increased phytoplankton concentrations can indirectly be responsible for presently observed loss of macrophytes coverage due to light limitation.

Abbreviations: cDOM:chromophoric dissolved organic matter; Chl *a*, chlorophyll *a*; daily*PFD*, integrated photon flux density (400–700 nm) per day (μmol photons m^{-2} d^{-1}); E_c, light compensation point (μmol photons m^{-2} s^{-1}); E_k, light saturation point (μmol photons m^{-2} s^{-1}); EU-WFD, Water Framework Directive of the European Union (GL 2000/60/EC); k_0, scalar light attenuation coefficient (400–700 nm), (m^{-1}); min*PFD*, integrated minimum photon flux density for macrophyte growth

Introduction

Macrophyte distribution and abundance is influenced by biological and environmental variables. Germination, growth and reproduction are dependent on light quality and quantity (Chambers & Kalff, 1985), interspecific competition (Madsen et al., 1991a, b; McCreary, 1991), nutrient availability (Blindow, 1992), herbivory (Painter et al., 1988; Lotze & Schramm, 2000), physical damage by bottom-feeding fish (carp *Cyprinus carpio*, Painter et al., 1988), intensive natural disturbance events such as storms or ice damage (Rybicki & Carter, 1986; Crowder and Painter, 1991), fluctuating water levels and water depth (Martin & Uhler, 1939; Spence, 1982; Blindow, 1992), water temperatures (Hutchinson, 1975), alkalinity and pH (Moyle, 1945; Hutchinson, 1970; Hellquist, 1980), salinity (Serrao et al., 1996; Orfanidis et al., 2000), aquatic pollution (Lovett Doust et al., 1994; Lewander et al., 1996), and composition of sediments (Hutchinson, 1975; Kautsky, 1991).

The reduction of macrophyte abundance and species variety, and especially of Characeae, has been reported for the lagoons of the southern Baltic Sea. In these ecosystems, Krause (1981), Walter (1981), Behrens (1982), Geisel (1986), Teubner (1989), Schiewer (1994), Schiewer et al. (1994) and Yousef et al. (1997a, b) reported on a loss, or even on the extinction, of some species (extinct: *Chara connivens*, *Lamprothamnium papulosum*; Bluemel et al., 2002). Eutrophication is supposed to be the main driving force and may cause an increase in underwater light attenuation through increased phytoplankton concentrations (Kautsky et al., 1986; Malm et al., 2001). Epiphyte cover will also negatively affect light availability of macrophytes and may also act as a competitor for nutrients (Kautsky, 1991; Lindblad & Kautsky 1992).

One of the main objectives of the EU-WFD is to achieve a 'good ecological state' for all surface waters including coastal and transitional waters a until 2015. A 'good ecological state' is defined as only slightly deviating from natural surface water body types undisturbed by anthropogenic impacts. Thus, ecologists have to solve the question how pristine communities of animals and plants were structured. Assessment of the pristine ecosystem conditions can be based on (a) the analysis of recent water bodies still in pristine conditions, (b) the use of historical, i.e. pre-industrial descriptions, (c) regression analysis of long-term monitoring data to times before data collection started, or (d) in a more pragmatic attempt by using earliest descriptions available, which may already fall into the period of anthropogenic influence of the ecosystem. Therefore we consider the latter attempt as not acceptable. For the inner coastal waters of the southern Baltic Sea, none of the above named possibilities seems to be sufficient alone. However, a combination of methods may achieve results consistent enough to develop a sound conceptual model of anthropogenic undisturbed macrophyte communities.

We combined the analysis of ecophysiological characteristics of macrophyte species recently occurring in the systems investigated, with trend analysis of abiotic main factors, in order to describe the reference conditions of submerged brackish-water vegetation communities. Herbarium records, as a substitute for missing historical descriptions, were used for the verification of modelled plant distribution.

Investigations of several regional macrophyte surveys and monitoring programs enabled us to describe the main factors which determine growth and distribution of macrophytes. These parameters are: salinity, light penetration, substratum, and ice coverage. Finally, considering the ecophysiological requirements of macrophytes these factors were subdivided in accordance to the physical and chemical descriptors listed in Annex II of the WFD (Table 1).

For marine (Schramm et al. 1988; Vogt & Schramm, 1991; Kukk & Martin, 1992; Burkholder et al., 1993; Hoffman, 1994; Kautsky, 1995; Drevs, 1995; Schramm, 1999), as well as for freshwater ecosystems (Barthelmes, 1984; Filbin & Barko, 1985; Gerbeaux & Ward, 1988; Dijk & Donk, 1991; Hough et al., 1991; Blindow et al., 1993; Kasprzak et al., 1995; Krienitz et al. 1996; Madsen et al., 1996; Broenmark & Weisner, 1999; Comin et al., 1999), altered light climate due to eutrophication is thought to be the most important variable affecting growth and distribution of macrophytes.

In this study we treated other abiotic factors of the study sites, such as salinity and currents, as a constant, oscillating in the same order of magnitude since pre-industrial times. Missing historical underwater light climate data were re-evaluated by a model based on recent measurements of water bodies in conditions comparable to reference conditions. Modelling of pristine underwater light conditions was done by recording light attenuation spectra during winter time, in an mesotrophic lagoon with today still low concentrations of humic substances, and low resuspension of sediments. Hence, phytoplankton concentration and their influence on underwater light climate was thought to be as low as in pristine lagoons. We then used surface light intensities of a reference year in order to calculate depth-dependent light doses for each lagoon. The results, in combination with species-dependent light requirements for growth (E_k-values), enabled us to compute pristine depth distribution limits for macrophytes. Finally the calculated model values were compared with data on light climate and macrophyte distribution collected in the 1970s, recent measurements (Yousef, 1999), and macrophyte distribution data derived from historical herbarium specimen from 1857–1861, kept at different German Universities.

Description of sites studied

The investigated semi-enclosed Bodden of the German Baltic Sea (54.5–54° N; 11.5–14° E) are shallow and brackish (Table 2). Mean depth varies between 1.8 and 5.8 m. Salinity is dependent on the quantity of inflow

Table 1. Annex II, System B, of the EU-WFD for the typology of the coastal waters. Factors influencing the growth and distribution of macrophytes in the lagoons of the Baltic Sea were divided in ecological relevant units. Salinity (psu), depth (% of surface irradiance).

	Factor	Type		Number of types
Obligatory	Salinity	0.5–3	β-Oligohaline	4
		3–5	α-Oligohaline	
		5–10	βb-Mesohaline	
		10–18	α-Mesohaline	
Optional	Depth	40%		4
		10%	Euphotic zone	
		1%		
		0.1%	Aphotic zone	
Optional	Substratum	Mud		
		Grain size	Sandy mud	
		(Kolp, 1966)	Muddy sand	7
			Sand	
			Gravel	
			Boulders	
			Blocks	
Optional		Ice coverage	Regularly destruction of	2
		Above-ground shoots not affected		

Table 2. Physico-chemical parameters of sampling sites (Correns, 1979; Schlungbaum, 1997; Umweltministerium, 1999).

Parameter	Salzhaff	Darß-Zingst-Bodden	Greifswald Bodden
Volume (km^3)	ca. 0.06	0.397	2.96
Area (km^2)	27	186.6	510.2
Catchment area (km^2)	271	1593	659.3
(rivers)	(Hellbach)	(Recknitz, Barthe, Körkwitz Bach, Saaler Bach)	(Ryck, Ziese)
Depth (m), mean (max.)	2.3 (9.5)	2 (12)	5.8 (13.5)
Long-term annual salinity (psu) mean (max./min.)	12.5 (19.6/7.8)	Saal 1–3 (4/0.5) Bodstedt 3-4 (7/1) Barth 5-6 (10/1.5) Grabow 8-12 (15/7)	6.2–7.8 (11.2/3.2)
Substratum type	Mud, sand, rarely coarse sand, stones	Mostly mud, fine sand, (rarely coarse sand, stones)	Mud, fine sand, coarse sand, stones
Nutrient status (1996/97)	Mesotrophic	Highly eutrophic	Eutrophic
Notes	Macrophyte-dominated	Steep gradient of salinity, high concentration of humic substances	Large lagoon, diverse substrates, major influence of the adjacent Baltic Sea

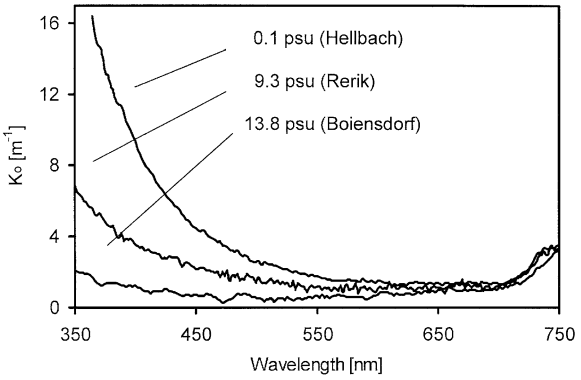

Figure 1. Attenuation spectra of water bodies at different sites in the Salzhaff Bodden on January 9th, 2001. Shown are attenuation spectra calculated from 6 underwater irradiance spectra measured between 0.1–1 m depth at Boiensdorf (outlet of the Salzhaff Bodden, 13.8 psu), Rerik Harbour (9.3 psu), and river Hellbach mouth (0.1 psu).

of fresh water from rivers and dynamics of exchange with the directly connected Baltic Sea. Average salinity of the Baltic Sea in front of the analysed lagoon systems varies between 16 and 7 psu, following a gradient from west to east.

Materials and methods

Computation of pristine underwater light climate

At present, dissolved organic matter (cDOM) and phytoplankton cells are the main light absorbing substances in the studied areas (Schubert et al., 2001). The high cDOM concentration in the water column of the Bodden mainly drains from the catchment area consisting of degraded fens and mires. Assuming that anthropogenic influences during the last centuries were responsible for the increase of cDOM concentrations in most of the ecosystems, we used the less influenced Salzhaff area as reference for measurements of underwater light attenuation. Here the catchment area was small, and the only freshwater inflow was the river Hellbach, a stream only moderately influenced by draining activities. The inflow from freshwater streams transporting cDOM was mixed with more saline water of the Baltic Sea, resulting in a dilution of the cDOM concentration in the Bodden towards the sea. Thus, the salinity gradient was used as an easy to measure approximation for cDOM towards the open Baltic Sea. As a simplification mixing of the waters, i.e. a change in cDOM concentration, was determined from long-term mean salinity, assuming a linear gradient of salinity from freshwater (100% cDOM) towards Baltic Sea water (0%, reference value of cDOM concentration). Light attenuation was measured on 9 January 2001, when water temperature was 4 °C and phytoplankton concentrations at a minimum. The results were verified by comparing measurements from earlier studies in the Darß-Zingst-Bodden-Chain and the Greifswald Bodden (Schubert et al., 2001; unpublished results of BMBF-project UV-MAOR, 1998), and with results of Lindner (1972).

At five sample locations in the Salzhaff we measured underwater irradiance with a high-resolution spectrophotometer (SR-9910, Macam Photometrics, Livingston, Scotland), equipped with a 9 m light guide and a spherical light collector of 0.7 cm diameter (Schubert et al., 1995). Total absorption of dissolved organic compounds was measured in an 1 cm quartz glass cuvette with a spectrophotometer (Lambda 2, Perkin-Elmer, Überlingen, FRG), after samples were filtered through glass fibre filters (0.4 μm pore size, GF 6, Schleicher & Schuell, Dassel, Germany). Fluorescence emission spectra of the filtrates were scanned in an 1 cm quartz glass cuvette with a spectrofluorometer (F 4010, Hitachi, Kyoto, Japan) in order to check for water-soluble pigments in the filtrate.

Ecophysiology of macrophytes and calculated depth limits

In a literature analysis we collected available data of ecophysiological tolerances for the macrophytes of interest (Bluemel et al., 2002). Combining the computed light attenuation coefficient (k_0) at the study-sites and values of daily surface light intensity (daily*PFD*) measured in 1998, we were able to calculate the depth in which light was sufficient for growth. As a prerequisite for growth in a given depth, we defined the species-specific light saturation point (E_k), available for at least ten hours per day, at seven consecutive days, as a minimum period of light availability (min*PFD*). Finally, the average of the calculated depth-limits of seven consecutive days were displayed versus time (weeks) throughout the reference year 1998.

$$\text{depth} = \frac{\ln(\text{daily}\,PFD) - \ln(\min PFD)}{k_0},$$

with

$$\min PFD = E_k \times 3600 \times 10.$$

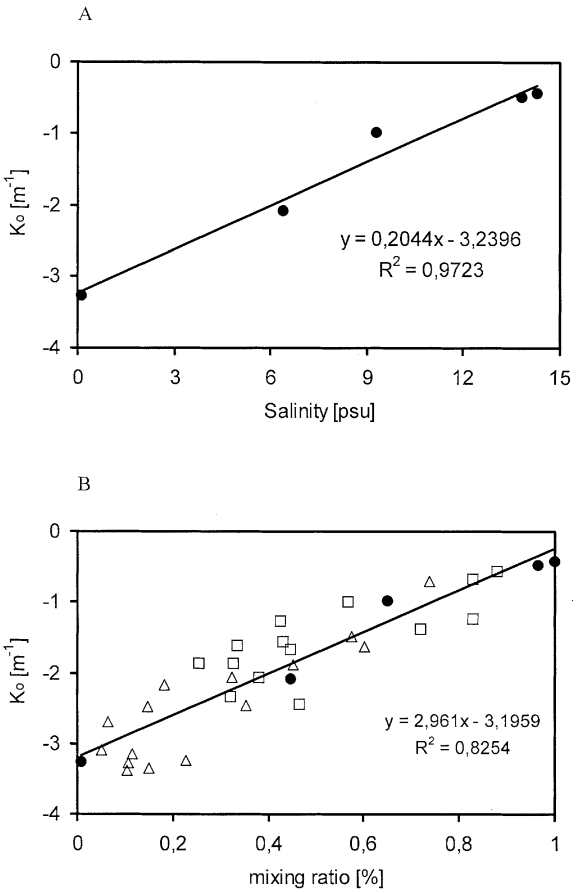

Figure 2. Scalar light attenuation coefficient k_0 at different sites in the Salzhaff (top), and correlation of k_0 vs. ratio of fresh- and Baltic Sea water in three Bodden systems (bottom). Symbols used: filled circles: Salzhaff Bodden, triangles: Darß-Zingst-Bodden-Chain, squares: Greifswald Bodden. Trend lines are calculated by the formula given in the figures.

Figure 3. Theoretical depth limits and observed depth limits of *Chara baltica* for the Bodstedt Bodden (top) and the Barth Bodden (bottom). Observed depth limits based on herbarial material collected by Holtz (1857–1861, broken line), data of Lindner (1972, thin line), and Yousef (1999, thick line). Light availability curves are representing the depth in which min*PFD* is available. Attenuation coefficients for calculation were taken from the model described here (vertical striped area), measurements of Lindner (1972, dotted area) and measurements of Schubert et al. (2001, grey area). Smoothing of area by adjacent averaging over a 9-week period.

Results

Light attenuation and pristine underwater light climate

Attenuation spectra of water sampled in the Salzhaff showed that turbidity decreased with increasing salinity. Site Boiensdorf, close to the Baltic Sea, had higher transparency than the river mouth Hellbach (Fig. 1), where the light attenuation caused by humic substances between 350–450 nm was severe. In these turbid waters UV-A and blue light was absorbed within few decimetres, and light available for photosynthesis was restricted to the 500–700 nm range.

Turbidity was obviously correlated with the dilution of cDOM containing freshwater from the Hellbach (Fig. 2A). A comparison with unpublished data of winter light attenuation measurements in the Greifswald Bodden and the Darß-Zingst-Bodden-Chain (BMBF-project UV-MAOR, 1998) showed that measurements in these three Bodden-systems were consistent. A linear regression of all measurements, therefore, was regarded as a sufficient approximation of the historical light attenuation (Fig. 2B).

Taking into account long term mean values of salinity in the Bodden systems and the adjacent Baltic Sea, we calculated light attenuation coefficients for the study-sites (Table 3). Results show that without anthropogenic impact depth of the euphotic zone was slightly less than mean depth only at the Saal-Bodden, which is characterised by high turbidity. In general eu-

Table 3. Long term mean salinity (psu), depth (m), calculated scalar light attenuation coefficient, k_0, (m^{-1}), and euphotic zone, 1% SI (m below surface) of Bodden systems, and long term mean salinity (psu) in offshore Baltic Sea (Correns, 1979; Schlungbaum, 1997; Umweltministerium, 1999).

Study area, Bodden	Mean salinity Bodden	Mean salinity adjacent Baltic Sea	Depth (max)	Depth (mean)	K_0	1% SI
Salzhaff	13.8	13.1	9.5	2.3	−0.16	28.1
Saal	3.2	10.9	4	2.2	−2.38	1.9
Bodstedt	4.7	10.9	10	1.9	−1.98	2.3
Barth	6.6	10.9	6.5	1.8	−1.47	3.1
Grabow	7.9	10.9	4.5	2.2	−1.12	4.1
Greifswald	7.7	8	13.5	5.8	−0.43	10.7

photic depth exceeded mean depth of the Bodden ecosystems. In the Salzhaff, which is characterised today by high transparency even during summer months, the computed euphotic zone exceeded maximum depth (Table 3).

Ecophysiology and theoretical depth distribution of macrophytes

We were able to calculate depth limits for several macrophytes according to published light saturation points and computed pristine light climate in the Bodden. Table 4 summarises theoretical depth limits and maximum vegetation period at 0.3 m depth. Considering light was as the only limiting factor, the Salzhaff Bodden could be covered completely and colonised by all macrophytes listed in Table 4. On the other hand, the Saal Bodden, with a high concentration of humic substances and mean depth of 2.2 m, should be populated only down to 1.3 m depth. *Ceramium rubrum*, a Rhodophyceae which was able to grow under very dim light, may find sufficient light at 0.3 m depth during the whole year. Other species with higher light demands, like *Ulva curvata*, could grow in the Saal Bodden at 0.3 m depth only during summer months (week 18–31).

Yousef (1999) studied photosynthesis, growth and distribution of *Chara baltica* and *Chara canescens* in the Bodden. The measurement of light intensity at the lower distribution limit of those species, together with laboratory growth experiments, showed, that *in situ* light intensities matched quite well laboratory based determination of E_k for growth. *In situ* light intensities at the lower distribution limit during vegetation period (May-September) was 47.6 ± 22% of SI (∼15 mol m^{-2} d^{-1}) for *C. canescens,* and 41.3 ± 21% of SI (∼13 mol m^{-2} d^{-1}) for *C. baltica*. In the laboratory, growth efficiency was found to be maximal at light intensities of 17.6 mol m^{-2} d^{-1} for *C. canescens* and between 17.6–22.6 mol m^{-2} d^{-1} for *C. baltica*. However, the E_k values for photosynthesis determined during the laboratory experiments were lower than E_k values for growth by approximately 50% for both species (Yousef, 1999). Combining the measured surface light intensity, computed light attenuation coefficients for Bodden waters, and physiological demands, we calculated maximum distribution depths for common macrophytes of the Bodden ecosystems. Upper distribution limit for all macrophytes was set to 0.3 m depth, because wind- and pressure-induced fluctuations of water level, grazing by waterfowl, as well as mechanical stress by waves and abrasion by ice coverage during severe winters, were described as natural limiting factors (Yousef, 1999; Malm et al., 2001). Calculations of light demands taken from *in situ* distribution patterns (Yousef, 1999) showed that light intensities at 0.3 m depth in the Bodstedt Bodden, were sufficient for growth of *Chara canescens* during the weeks 13 to 32. For *C. baltica*, which had lower light demands, the vegetation period lasted from week 12 to 34. Calculated with *in situ* light demands, the maximum depth limits were −0.4 m for *C. canescens* and −0.5 m for *C. baltica* in summer. They were significantly smaller than the depth limits calculated from light demands based on E_k for photosynthesis in laboratory experiments (−0.73 m for *C. canescens* and −0.78 m for *C. baltica*, Table 4).

Leukart & Lüning (1994) reported on light demands of *Ceramium rubrum* and determined an E_k of 55 μmol m^{-2} s^{-1}. The pristine light conditions of the

Table 4. Calculated depth limits according to E_k (μmol m^{-2} s^{-1}) and computed pristine k_0 of Bodden. Maximum depth of distribution [m below surface] and light period with intensities $\geq E_k$ at upper distribution limit, listed in brackets (week of the year). If no period is listed, light at -0.3 m is always $\geq E_k$.

Bodden	*Chara baltica*		*Chara canescens*		*Potamogeton pectinatus*		*Ulva lactuca*		*Ulva curvata*	
Salzhaff	9.48		9.11	(3–48)	7.90	(5–47)	16.89		5.41	(7–43)
Saal	0.65	(11–38)	0.63	(11–38)	0.54	(12–35)	1.16	(5–48)	0.37	(19–30)
Bodstedt	0.79	(10–40)	0.75	(9–39)	0.65	(5–37)	1.39	(4–50)	0.45	(16–32)
Barth	1.05	(9–42)	1.01	(3–42)	1.07	(9-41)	1.88	(2–51)	0.60	(13–33)
Grabow	1.38	(8–43)	1.33	(8–43)	1.15	(9–41)	2.46	(1–51)	0.79	(12–35)
Greifswald	3.61	(5–47)	3.47	(5–46)	3.01	(6–45)	6.44		2.06	(9–40)
E_k	235		250		305		70		7.90	
Author	Laboratory, Yousef, 1999		Laboratory, Yousef, 1999		Menendez & Sanchez, 1998		Fortes & Lüning, 1980		Rosenberg & Ramus, 1982	
Bodden	*Fucus serratus*		*Fucus vesiculosus*		*Ceramium rubrum*		*Zostera noltii*		*Ceramium glanduliferum*	
Salzhaff	14.71		9.11	(3–48)	18.37		7.90	(5–47)	4.46	(8–41)
Saal	1.01	(6–45)	0.63	(11–38)	1.26	(4–51)	0.54	(12–35)	–	–
Bodstedt	1.21	(5–46)	0.75	(9–39)	1.51	(2–51)	0.65	(5–37)	0.37	(20–39)
Barth	1.63	(5–48)	1.01	(3–42)	2.04	(1–51)	1.07	(9–41)	0.49	(15–32)
Grabow	2.14	(4–51)	1.33	(8–43)	2.67		1.15	(9–41)	0.65	(13–33)
Greifswald	5.61		3.47	(5–46)	7.00		3.01	(6–45)	1.70	(9–38)
E_k	100		250		55		305		551	
Author	Bird et al., 1979		Niemeck & Mathieson, 1978		Leukart & Lüning, 1994		Vermaat & Verhagen, 1996		Stirk et al. 1995	

Greifswald Bodden would therefore enable growth at the upper limit (-0.3 m) during the whole year and a maximum depth limit of 7 m during summer. Macrophytes with higher light demands, like *Zostera noltii* ($E_k = 305$ μmol m^{-2} s^{-1}, Vermaat & Verhagen, 1996), would absorb sufficient light intensities during the weeks 6 to 45, and reach a maximum depth limit of -3 m.

The validity of the computation of pristine depth distributions was checked by comparing them with published data. The oldest data were herbarium records sampled by Holtz between 1857 and 1861, then came Lindner (1972), Küster et al. (1998), Yousef (1999) and most recently Schubert et al. (2001). A comparison of observed depth limits between 1861 and 1998 showed a decrease in maximum depth limit for *Chara baltica* in the Bodstedt and Barth Bodden (Fig. 3). Moreover, if maximum instead of average salinity values of 1972 are used (9.96 psu), calculated pristine water transparency is increased ($k_0 = -0.57$ m^{-1}) and macrophytes should be able to grow in deeper zones (Fig. 4). Depth limits are then increased to -1.94 m in case of *Chara baltica*, if light demands determined by *in situ* measurements of Yousef (1999) are used.

Discussion

Several environmental, species-dependent factors are not necessarily connected to light, but they may influence the distribution of macrophytes. In our model, we based the calculations on theoretical assumptions of pristine underwater light climate and existing ecophysiological data of only a few, selected species. Higher transparency of the Bodden would enable all macrophytes studied to grow in deeper zones, compared to actual distribution patterns, or reported depth limits in 1972. Herbarium records were found to support our calculations, even though we did not take into account other biotic or abiotic factors besides light. Several models already exist, which try to predict growth and biomass, e.g. for *Zostera marina* (Wetzel & Neckles, 1986), *Potamogeton pectinatus* (Madden

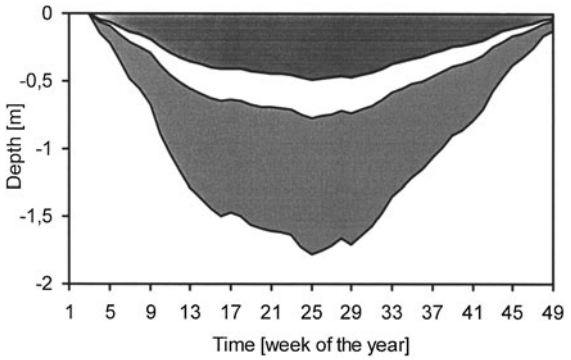

Figure 4. Theoretical depth limits of *Chara baltica* for the Bodstedt Bodden, based on light demands from *in situ* observations (Yousef, 1999). Weekly light availability was based on k_0, either computed from 4 psu salinity (grey area), 7 psu (dotted area) or 10 psu (vertical striped area). *In situ* salinity was measured by Lindner (1972) giving an average of 4.7 psu with maximum values up to 9.6 psu and minimum values down to 2 psu. Smoothing of area by adjacent averaging over a 9 week period.

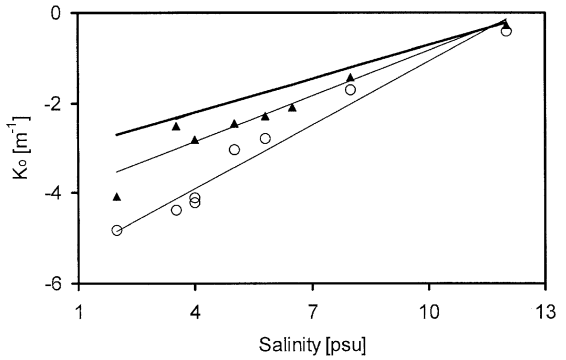

Figure 5. Linear regression of calculated light attenuation coefficient k_0 (m^{-1}) for pristine conditions (thick black line), calculated by means of the model described above (comp. also with Fig. 3), and *in situ* measurements of Lindner 1972 (filled triangles) and Schubert et al. 2001 (measured 1998, open circles), as a function of salinity for the Darß-Zingst Boddenchain. Thin lines represent the trends for the measurements of Lindner ($y = 0.3365x - 4.2064$, $R^2 = 0.9287$, $n = 8$) and Schubert et al. 2001 ($y = 0.4704x - 5.7855$, $R^2 = 0.962$, $n = 8$).

& Kemp, 1996), *Halodule wrightii* (Fong & Harwell, 1994; Fong et al., 1997, Burd & Dunton, 2001).

As a possible explanation for limited distribution of macrophytes in the Bodden we favour the successive deterioration of underwater light availability, which is shown in Fig. 5. In this Figure, k_0-values measured by Schubert et al. (2001) and Lindner (1972) along the Darß-Zingst Bodden were plotted against salinity and compared with the results of the calculations done with the underwater light model described above. The results obtained by this model fit quite well with the succession of the lower depth limit for growth of *Chara baltica* in the Bodstedt Bodden as well as the Barther Bodden, if applied to the herbarium records of Holtz, sampled between 1857 and 1861 (Fig. 3).

Modelled maximum depth distribution are not always in accordance to depth distribution found *in situ*. Calculations with *in vitro* light intensities usually result in deeper limits, because growth conditions in the laboratory are kept ideal, i.e. light is the only limiting factor. On the other hand, usually it is difficult to measure *in situ* light intensity at the depth limit during the whole growth season. Insufficient determination of light regime, therefore, may be a reason for the discrepancy between calculated light demands of macrophytes in the laboratory and *in situ*. Also, observed depth limits *in situ* include other factors besides light, like grazing, infections, competition, temperature regime or nutrient availability, which are usually neglected in laboratory growth experiments. However, our results show that calculations are suited to verify historical data, and to predict a possible increase of depth limits as a consequence of increased transparency, e.g. found by Kautsky et al. (1986) and others. Calculated pristine underwater light climate and measurements of attenuation coefficients (Fig. 4) showed a correlation of anthropogenic eutrophication with available underwater light. Historical records and descriptions of maximum depth distribution during the last decades support this thesis (Fig. 3). Although light is not the only factor for distribution of plants, it may be the most important one. Especially in the shallow and well mixed Bodden ecosystems, CO_2-availability and nutrients are not likely to limit growth. Long-term changes in salinity therefore might change macrophyte depth distribution by influencing both, the physiological state of the individuals as well as underwater light availability.

Our calculations for a few selected macrophytes can be regarded as a conceptual model of maximum depth limits of the physical and chemical properties of a Bodden in Southern Baltic Sea at high ecological status. Our model is not able to determine differences between pristine and present ecological states, i.e. actually we are not able to characterise the pristine ecological state in terms of depth-dependent plant community structure and percentile macrophyte coverage from historical records alone. The transition between a very good and a bad ecological status remain unknown, although some theories exist (e.g. Schiewer, 1994; Schiewer et al., 1994; Scheffer, 1998). Results show, that pristine maximum depth limits were deeper, and present depth limits are shallower than

in the 1970s. Loss of macrophyte coverage in deeper zones can be regarded as a deterioration of the ecological status, even when in shallow depths community structure is not altered. For a model of the whole Southern Baltic lagoons more species-dependent data on eco-physiological tolerance are necessary. Therefore, one future aim of our project is to measure those changes of growth and photosynthesis parameters, and to incorporate them into our model of distribution. Currently the main problem is our lack of information about the relationship between E_k and E_c, and insufficient information about species-dependent minimum light demands *in situ*.

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Active carbon uptake in *Laminaria digitata* and *L. saccharina* (Phaeophyta) is driven by a proton pump in the plasma membrane

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Key words: acetazolamide, carbon uptake, DIDS, erythrosin B, *Laminaria*, proton pumps, vanadate

Abstract

Mechanisms for inorganic carbon acquisition in *Laminaria digitata* (Hudson) Lamour and *L. saccharina* (L.) Lamour from the Swedish west coast were studied in pH-drift experiments, using several inhibitors for different types of carbon uptake across the cell membrane. Throughout the experiments total carbon decreased in concert with a pH increase while alkalinity stayed relatively stable. Addition of acetazolamide had a strong inhibitory effect on the carbon uptake rate in *L. digitata* and the anion exchange protein inhibitor DIDS had a small inhibitory effect above pH 9.5. These results indicate that carbon uptake in *L. digitata* depends on the presence of an external carbonic anhydrase while direct bicarbonate uptake may contribute at high pH. These two mechanisms have previously been shown to occur in *L. saccharina*. We show that two inhibitors of H^+-ATPases, vanadate and erythrosin B, also decreased carbon uptake rates in both *Laminaria* species. The effect of erythrosin B was immediate and it probably acts on the outside of the cell membrane. Contrarily, vanadate needs to be transported into the cell, where it competes with the phosphate from ATP for the aspartic acid phosphorylation site on the plasma membrane P-type H^+-ATPase. Therefore, 1–2 h of pH drift were usually required before an inhibitory effect became apparent. Additional experiments with P-enriched and P-starved *L. saccharina* corroborated this process. Based on these results we suggest that the investigated *Laminaria* species, besides external carbonic anhydrase and DIDS-sensitive anion exchange, also possess a mechanism for the active uptake of carbon, which is dependent on plasma membrane P-type H^+-ATPase activity. This paper also reports on the buffering capacity of the inhibitors when used in natural seawater, an aspect that has been neglected in previous studies.

Abbreviations: ANOVA, analysis of variance; AZ, acetazolamide; CA, carbonic anhydrase; CV, coefficient of variance; DIC, dissolved inorganic carbon; DIDS, 4,4′-diisothiocyanostilbene-2,2′-disulfonate; EB, erythrosin B; MLD, sodium molybdate; NSW, natural seawater; SE, standard error of the mean; STD, standard deviation; BT, BIS-TRIS propane; TRIS, Trizma base; VAN, sodium orthovanadate.

Introduction

In oceanic seawater with a salinity of 35 psu the concentration of dissolved inorganic carbon (DIC) is approximately 2.1 mM when in equilibrium with air. At a natural pH of about 8 the major part of the DIC is in the form of bicarbonate (HCO_3^-), and only about 12 μM is present as dissolved CO_2 gas (Stumm & Morgan, 1996). Carbon dioxide, being an uncharged molecule, readily diffuses into an algal cell and is directly available for carbon fixation, while the lipid bilayer of the plasma membrane imposes a barrier for diffusion of HCO_3^- (Gutknecht et al., 1977). In a submersed environment with high insolation, dense algal biomass and low water exchange, CO_2 may become limiting for photosynthesis as pointed out already by Tseng & Sweeney (1946). Therefore, a capacity to utilize also HCO_3^- for carbon fixation, in addition to

CO_2, is of great advantage to aquatic photosynthetic organisms. Several macroalgae have been shown capable of using HCO_3^- as a carbon source (Maberly, 1990; Larsson & Axelsson, 1999).

When CO_2 is withdrawn from the ambient seawater during photosynthesis it will continuously be replenished from the HCO_3^- pool. However, the spontaneous dehydration of HCO_3^- into CO_2 is a rather slow process unless catalysed by carbonic anhydrase (CA), a Zn metallo-enzyme which is ubiquitous in photosynthetic organisms (Badger & Price, 1994). The presence of periplasmic CA in algae has been shown with the aid of the inhibitors acetazolamide (AZ; Moroney et al., 1985; Granbom & Pedersén, 1999) and dextran-bound sulfonamide (Haglund et al., 1992a, b), two substances which do not, or only extremely slowly, penetrate into the algal cells. The HCO_3^- in the ambient water thus acts as an external reservoir for CO_2, and the newly formed CO_2 in the periplasmic space can diffuse or be actively transported into the cell.

There is also evidence for a direct uptake of HCO_3^- in algae, through exchange of HCO_3^- for Cl^- or OH^- ions mediated by a protein similar to the red blood cell anion exchanger (AE1). In *Ulva* sp., HCO_3^- uptake is inhibited by 4,4′-diisothiocyanostilbene-2,2′-disulfonate (DIDS; Drechsler et al., 1993), a classical probe of anion exchange in red blood cells. A membrane-located protein in *Ulva* sp. was recognised by antibodies raised against the human AE1 (Sharkia et al., 1994). The inhibitory effect of DIDS on algal photosynthesis at high pH has been shown for several other algal species as well (Andría et al., 1999; Granbom & Pedersén, 1999; Larsson & Axelsson, 1999).

Active (energy-consuming) carbon uptake in macroalgae has been suggested by several authors (e.g. Lucas, 1983). However, studies providing experimental evidence for active uptake are scarce for macroalgae (but not for cyanobacteria and microalgae). Mimura et al. (1993) showed that perfusion of cells of the freshwater characean *Chara corallina* with ATP-depleted medium or medium containing vanadate inhibited photosynthetic ^{14}C-fixation both when inorganic carbon in the external medium was supplied as CO_2 (pH 5.5) or HCO_3^- (pH 8.5). In contrast, photosynthesis of isolated chloroplasts and C-fixation of cells supplied with ^{14}C internally were insensitive to VAN or internal ATP-depletion. This indicates that transport of both CO_2 and HCO_3^- in *C. corallina* occurs at the plasma membrane and that it involves the activity of H^+-ATPases. Active CO_2 or HCO_3^- uptake in living macroalgae has previously been shown by pH-drift experiments for the red alga *Coccotylus truncatus* (Snoeijs et al., 2002) and for the green algae *Cladophora glomerata* and *Chara tomentosa* (Choo et al., 2002; Ray et al., 2003), but not for brown algae. Some brown algae, mainly species belonging to the family Fucaceae, take up carbon with a concomitant decrease in alkalinity as shown by Axelsson & Uusitalo (1988) and Uusitalo (1996) and they hypothesised that the algae excreted protons to the ambient seawater during photosynthesis. A decrease in photosynthetic oxygen evolution as a result of the addition of proton buffers to the medium also suggests the involvement of proton excretion in carbon uptake (Price & Badger, 1985; Axelsson et al., 2000).

In the present study we investigated the carbon uptake strategies of two *Laminaria* species with special emphasis on the occurrence of active transport. We hypothesized that the mechanism involving proton excretion is the occurrence of a pH gradient ('proton motive force'; Taiz & Zeiger, 1998) across the cell membrane energized by a H^+-ATPase (proton pump). This enzyme has not previously been shown to be involved in carbon uptake in brown algae. The proton motive force is assumed to drive secondary transports and in the case of carbon uptake this would be the transport of CO_2 or HCO_3^- into the cell. Our approach was to directly block the H^+-ATPases that create the proton motive force with suitable inhibitors known to act on the activity of plasma membrane H^+-ATPases. A range of compounds is known to inhibit proton pumps, but none of them is specific for plasma membrane H^+-ATPases. Vanadate (VAN), which is commonly used as an inhibitor of plasma membrane P-type H^+-ATPases (Gilmour et al., 1985; Michelet & Boutry, 1995) also inhibits phosphatases (Gallagher & Leonard, 1982). Molybdate (MLD) is an inhibitor of phosphatase activity, and can thus be used in combination with VAN to rule out indiscriminate VAN inhibition of phosphatases. Besides VAN in combination with MLD, we used another inhibitor of proton pumps, erythrosin B (EB), which acts on both plasma membrane P-type H^+-ATPases and NO_3^--sensitive tonoplast ATPases (Cocucci, 1986; Cocucci & Marré, 1986; Beffagna & Romani, 1988). EB is inactive on phosphatases and scarcely active against mitochondrial and thylakoid ATPases. If an alga depends on the activity of plasma membrane H^+-ATPases for its carbon uptake, one would thus expect

its photosynthetic activity to be negatively affected by VAN and EB, but unaffected by MLD.

Materials and methods

The algal material

Specimens of *Laminaria digitata* (Hudson) Lamour and *Laminaria saccharina* (L.) Lamour were collected on the Swedish west coast. Until use, they were kept in tank culture at 8 °C in natural seawater of salinity 33 psu (NSW) from the sampling area with Provasoli nutrient enrichment (Provasoli, 1968) and illuminated by white fluorescent light at a photon fluence rate of 45 μmol photons m^{-2} s^{-1}. The water was exchanged every second week and Provasoli nutrient enrichment was added weekly. One day prior to the start of the experiments pieces of 1.0–1.5 g fresh weight (FW) of frond tissue were cut out, placed in NSW, and bubbled with air until use. In the experiments with phosphate-enriched and phosphate-starved *L. saccharina*, the tissue pieces were prepared one week in advance and kept in 2 l of NSW with Provasoli nutrient medium with and without phosphate, respectively.

Inhibitors

The inhibitors used were 0.2 mM AZ, 0.2 mM DIDS, 0.2 mM VAN, 0.2 mM MLD and 4 μM EB. AZ was dissolved in a small volume (less than 0.5 ml) of 0.5 M NaOH and was then diluted with seawater to the proper concentration. At a concentration of 0.2 mM and in the pH range 3–13, VAN is present in two forms, $H_2VO_4^-$ or HVO_4^{2-}, depending on pH (Harris, 1999, Fig. 29-4), which are the active forms for inhibition of P-type ATPase. VAN was dissolved directly in seawater at the concentration used in the experiment. A 100× stock solution of EB was prepared by dissolving EB in 95% ethanol. One ml EB stock solution was then added to 100 ml of seawater for experimental use, to reach a concentration of ethanol in the incubation medium of 1%. In the experiment with EB, 1% ethanol was added to the control treatment to exclude an effect of ethanol on photosynthesis. To compare the buffer capacity of VAN with that of a proton buffer, 0.2 mM Trizma base (TRIS, pKa = 8.1, Sigma) was used to study the rate of pH change due to absorption of H^+.

Buffer capacity of seawater

To be able to use the inhibitors of carbon uptake correctly, it is necessary to determine their possible effect on the buffer capacity of NSW. The buffer capacities of filtered NSW with and without 0.2 mM AZ, 0.2 mM VAN, 0.2 mM DIDS, 1 mM MLD, 0.2 mM TRIS or 0.2 mM BIS-TRIS propane (BT, $pKa_1 = 6.8$, $pKa_2 = 9.0$, Sigma) were determined by titration of 50-ml samples with 0.1 M NaOH, using an automatic titrator (719S Titrino, Metrohm^{TM}). Titration curves were made for the pH interval 6.50–10.00, with pH recordings for each 0.01 to 0.05 pH unit. Three replicate samples were measured for each treatment. The buffer capacity was calculated as the number of moles of OH^- added to achieve one pH unit of pH change and then expressed as titer slope in mM H^+ pH $unit^{-1}$.

pH, alkalinity and DIC

Pieces of *L. digitata* were placed in Erlenmeyer flasks containing 65 ml NSW with or without inhibitor. In experiments with inhibitors, the pH of the seawater was adjusted to the pH of NSW (∼7.8). The flasks were sealed gas tight with rubber stoppers and placed on a shaker table under illumination by daylight fluorescent tubes (100 μmol photons m^{-2} s^{-1}) at 12 °C. Every 1.5 h, three flasks were randomly taken out and the seawater was analyzed for pH, alkalinity and total carbon concentration (DIC). Alkalinity was measured using a back-titration method following Almgren et al. (1983). A 50-ml seawater sample was combined with 6.0 ml of 0.025 M HCl, boiled for 5 min., and then cooled on ice under bubbling with carbon-free air. The sample was titrated with 0.020 M NaOH to pH 6.00 using an automatic titrator (719 S Titrino, Metrohm^{TM}, and total alkalinity was calculated as $[H^+]_{added}$–$[OH^-]_{added}$. DIC was measured using a modified version of the methods described by Bidwell & McLachlan (1985) and Lignell & Pedersén (1986). Seawater samples of 1.0 ml were withdrawn from the incubation flasks through the rubber stopper with a syringe, and injected into 15-ml vials containing 1 M HCl. The vials were equipped with screw caps and silicone septa, and had prior to the analysis been equilibrated with carbon-free air twice. After injection of the samples, the vials were allowed to equilibrate for at least 30 min. with intermittent shaking, to ensure that all dissolved carbon species in the seawater sample were transferred into CO_2 gas in equilibrium with the gas phase in the vials. Gas samples were then withdrawn through the septum and analysed for

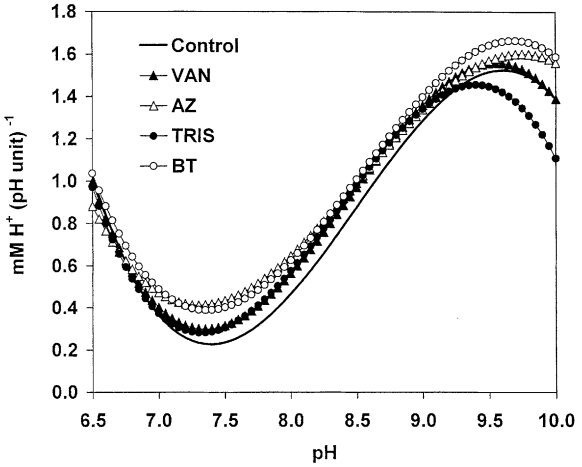

Figure 1. Trigonomal regression curves for the buffer capacity between pH 6.5 and 10.0 of NSW without additions (control, $R^2 = 0.96$) and NSW with 0.2 mM VAN ($R^2 = 0.97$), 0.2 mM AZ ($R^2 = 0.92$), 0.2 mM TRIS ($R^2 = 0.93$) or 0.2 mM BT ($R^2 = 0.96$). DIDS, EB and MLD did not increase the buffer capacity of NSW.

CO_2 content by injection into an IRGA (Infra Red Gas Analyser 225-2B-SS, ADC Ltd., England). The DIC content in the seawater samples was determined by comparison with freshly prepared HCO_3^- standard solutions.

pH-drift experiments

At the start of all pH-drift experiments, the pH of the NSW incubation medium was set to a fixed value of 6.3. The setup of the pH-drift experiments followed the design of Granbom & Pedersén (1999). The *Laminaria* pieces were rinsed in NSW with or without inhibitor adjusted to the proper start pH, and placed in Erlenmeyer flasks. The flasks were filled with 65 ml seawater medium and gas-tight sealed with rubber stoppers. A pH electrode (PHC 3006-9, Radiometer^{TM}) was inserted into each flask fitted through a hole in the stopper. The four pH electrodes were connected to two pH meters (PHM 250 Ion analyser, Radiometer^{TM}) and calibrated against three standard buffers (Radiometer^{TM} buffers pH 4, 7 and 10) before the start of each single run. In order to secure a continuous gas exchange between the algal material and the medium, the water was homogenized with magnetic stirrers and stirrer bars in the flasks. The stirring was separated from the alga with a plastic mesh above the stirrer bar to prevent the algal tissue from mechanical damage. Illumination (85 µmol photons m^{-2} s^{-1}), was provided by daylight fluorescent tubes on each side of the experimental setup. The temperature during the experiments was 6 °C. The pH in the experimental flasks was recorded every minute with a computerised pH logger system (Phlog1A, Topgallant, LLC, Salt Lake City, USA). Each experiment was carried out for 12 h during three consecutive days. On day 1, the algae were incubated in NSW (control). After this, the algae were rinsed and placed in NSW under aeration without illumination for 12 h. On day 2, the procedure was repeated but with the addition of an inhibitor or buffer to the medium (treatment). The algae were then thoroughly rinsed, and after a 12 h recovery period the algae were rinsed again and another incubation was performed with NSW on day 3 (postcontrol). In two of the experiments with *L. saccharina*, the algae were allowed to recover for three days after the postcontrol run, and a fourth incubation in NSW was conducted on day 7 (post-postcontrol). The experiments were always performed with three or four replicates.

Calculations and statistics

Polynomal regression analysis with pH as predictor variable and measured titer slope in µM H^+ pH $unit^{-1}$ as response variable was carried out for the pH interval 6.50–10.00 for each treatment. The regression equations were used to calculate µM H^+ pH $unit^{-1}$ (a) for each 0.01 pH unit between pH 6.50 and 10.00. A model for calculating pH, temperature, salinity, alkalinity and total DIC equilibria (D. Turner, Göteborg) was used to calculate µM DIC pH $unit^{-1}$ (b) for the NSW of 34 psu at 24 °C and alkalinity 1.25 mM (=NSW with pH adjusted to 6.5 with HCl) for each 0.01 pH unit between pH 6.50 and 10.00. The % decrease in the rate of pH change due to the absorption of H^+ was calculated for each treatment ($a \times 100\%/(a + b)$) for each 0.01 pH unit between 6.50 and 10.00. Polynomal regression analysis with pH as predictor variable and measured rates of pH change (pH unit kg FW^{-1} min^{-1}) as response variable was carried out for each treatment. The regression equations were used to calculate the rate of pH change for each 0.01 pH unit between 6.50 and 10.00 with the help of the % decrease in the rate of pH change due to the absorption of H^+, the effect of each treatment was subdivided into a buffering effect and an inhibition effect, expressed as pH unit kg FW^{-1} min^{-1} and µM DIC kg FW^{-1} min^{-1} for each 0.01 unit of pH change. Statistics were performed with the programme MINITAB^{TM}, Version 12.1. Significance was accepted at $p < 0.05$.

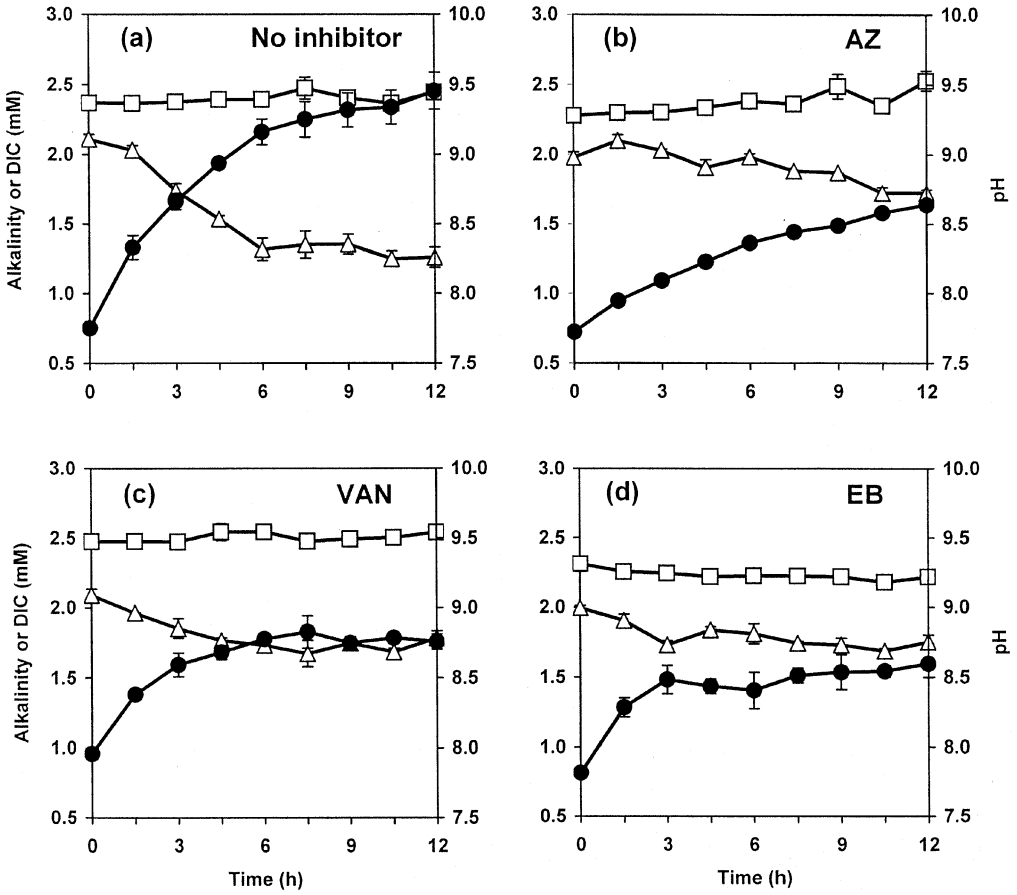

Figure 2. Changes in pH (circles), alkalinity (squares) and DIC concentrations (triangles) in the seawater medium, following photosynthesis of *Laminaria digitata* in (a) NSW without any addition, (b) NSW with addition of 0.2 mM AZ, (c) with addition of 0.2 mM VAN, and (d) with addition of 4 μM EB. Error bars indicate SE, $n = 3$. In symbols where error bars are missing, these are hidden behind the symbol due to the small SE.

Results

Buffer capacity

Addition of AZ, VAN, TRIS or BT increased the buffer capacity of NSW, with the exception of TRIS addition above pH 9.25 where the buffer capacity was lower than the control (Fig. 1). DIDS, EB or MLD did not increase the buffer capacity of NSW. The buffer capacity of NSW + 0.2 mM TRIS followed that of NSW + 0.2 mM VAN between pH 6.50 and 9.00, and the buffer capacity of NSW + 0.2 mM BT followed that of NSW + 0.2 mM AZ. The R^2 values of the regressions with pH as predictor variable and titer slope as response variable were between 0.92 and 0.97.

pH, alkalinity and DIC

The pH increased with time and the final pH after 12 h of incubation was 9.5 in the control, 8.6 with addition of 0.2 mM AZ, 8.8 with 0.2 mM VAN, and 8.6 with 4 μM EB (Fig. 2). The alkalinity (mean ± STD, $n = 27$) was 2.40 ± 0.06 mM in the control, 2.37 ± 0.10 mM in the AZ treatment, 2.50 ± 0.05 mM in the VAN treatment and 2.24 ± 0.06 mM in the EB treatment. There was no significant change in alkalinity with time in the control (ANOVA, $p = 0.360$), the VAN treatment ($p = 0.389$) or the EB treatment ($p = 0.241$), but in the AZ treatment alkalinity slightly increased with time during the experiment ($p = 0.006$). The DIC in the seawater medium was initially 2.10 mM and significantly decreased as photosynthesis proceeded down to 1.26 mM in the control, 1.72 mM in the AZ treatment, 1.78 mM in the VAN

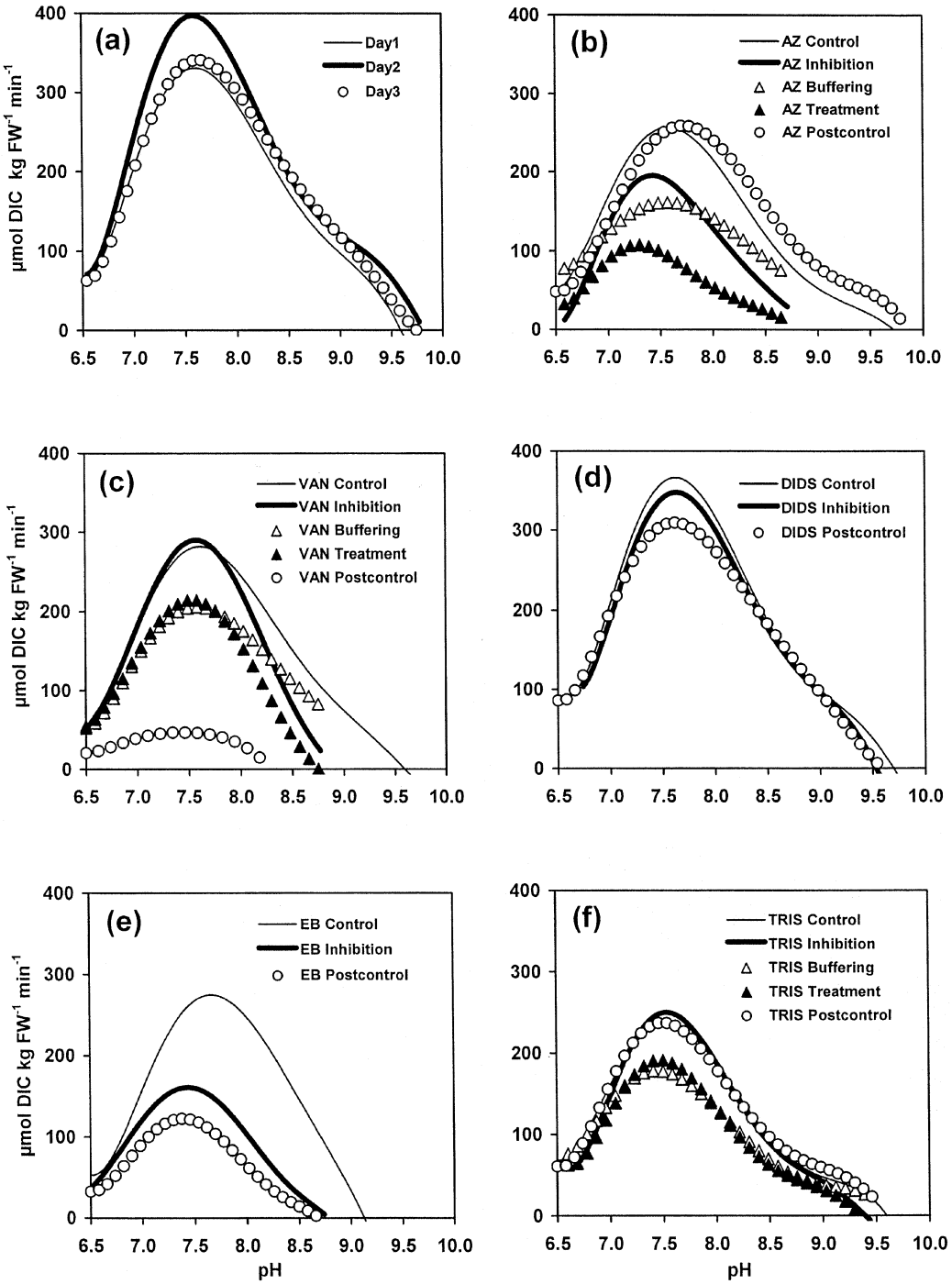

Figure 3. Results of the pH-drift experiments with *Laminaria digitata* presented as DIC uptake rates calculated for each 0.01 pH unit between 6.5 and 10.0. Each experiment includes three measuring days for control (without inhibitor or buffer), treatment (with inhibitor or buffer) and postcontrol (after rinsing off the inhibitor or buffer), respectively. The treatment is also subdivided into a buffering effect and an inhibition effect. Treatments: (a) reference without any inhibitor or buffer, (b) 0.2 mM AZ, (c) 0.2 mM VAN, (d) 0.2 mM DIDS, (e) 4 µM EB, (f) 0.2 mM TRIS.

Table 1. pH compensation points (± SD) of *Laminaria digitata* ($n = 4$), for the reference treatment and treatments with 0.2 mM AZ, 0.2 mM VAN, 4 mM EB, 0.2 mM DIDS, 1 mM MLD and 0.2 mM TRIS.

Inhibitor/buffer	Control	Treatment	Postcontrol	Post-postcontrol
Reference	9.65 ± 0.07	9.78 ± 0.04	9.77 ± 0.05	–
AZ	9.75 ± 0.05	8.72 ± 0.06	9.80 ± 0.07	–
VAN	9.68 ± 0.04	8.78 ± 0.13	8.22 ± 0.13	–
EB	9.14 ± 0.04	8.75 ± 0.04	8.70 ± 0.05	8.79 ± 0.12
DIDS	9.78 ± 0.03	9.60 ± 0.04	9.55 ± 0.02	–
MLD	9.61 ± 0.17	9.71 ± 0.07	9.78 ± 0.04	–
TRIS	9.64 ± 0.09	9.46 ± 0.07	9.53 ± 0.05	–

Table 2. pH compensation points (± SD) of *Laminaria saccharina* ($n = 4$), for phosphate-enriched material treated with 0.2 mM VAN (VAN +P), phosphate-starved material treated with 0.2 mM VAN (VAN −P), and normally cultivated material treated with 4 μM EB or 1 mM MLD.

Inhibitor/buffer	Control	Treatment	Postcontrol	Post-postcontrol
VAN +P	9.81 ± 0.08	9.45 ± 0.05	8.79 ± 0.11	–
VAN −P	9.86 ± 0.06	8.91 ± 0.32	8.03 ± 0.78	9.50 ± 0.27
EB	9.31 ± 0.07	8.45 ± 0.06	8.46 ± 0.06	–
MLD	9.70 ± 0.04	9.88 ± 0.03	9.93 ± 0.05	–

treatment, and 1.75 mM in the EB treatment (ANOVA, $p < 0.001$ for all treatments).

pH compensation points

In the control treatment of the pH-drift experiments (day 1), *L. digitata* normally reached a pH compensation point between 9.61 and 9.78 (Table 1) and *L. saccharina* between 9.70 and 9.86 (Table 2). However, in the controls of the EB treatment, the final pH was 9.14 in *L. digitata* and 9.31 in *L. saccharina* (Tables 1, 2). Probably, this was caused by the addition of 1% ethanol. The reference experiment, with *L. digitata* incubated in NSW without inhibitor for three days in a row, showed that the alga was not inhibited by the pH-drift experiment *per se* (Fig. 3a). On the contrary, a small enhancement of the carbon uptake during day 2 and 3 was recorded as an increase in the pH compensation point of 0.12–0.13 pH units (Table 1, *t*-test, $p < 0.05$). In *L. digitata*, the treatment with MLD did not affect the level of the pH compensation point (*t*-test, $p > 0.05$), but it was significantly lowered with the addition of AZ, VAN, EB, DIDS and TRIS (*t*-test, $p < 0.05$). The compensation points of the postcontrol treatments of AZ and TRIS were not significantly different from the control (*t*-test, $p > 0.05$), but significantly lower compensation points were obtained for VAN, EB and DIDS (*t*-test, $p < 0.05$). These results suggest that VAN was still active inside the cell, and that EB and DIDS could not be washed away from the cell membrane by rinsing the algae with NSW. For *L. saccharina* the same results as for *L. digitata* were obtained for MLD and EB. The experiment with P-enriched and P-starved *L. saccharina* showed only a slight decrease in the compensation point for the P-enriched algae (from 9.81 to 9.45), but a stronger decrease (from 9.86 to 8.91) in the P-starved algae. The same was still reflected in the postcontrol compensation points of 8.79 and 8.03 for these treatments, respectively. This indicates that the effect of VAN was significantly stronger when access to phosphate was limited. The pH compensation points of the post-postcontrols for VAN and EB (Tables 1, 2) show that VAN had almost disappeared from the cells after 3 days of recovery, while EB-treated algae were still inhibited.

DIC uptake rates

The DIC uptake rates in the controls of *L. digitata* increased from pH 6.5 until ca. 7.6 where they reached maxima of 235–395 μmol DIC kg FW^{-1} min^{-1}, and then decreased to zero at the pH compensation points (Fig. 3). The differences in DIC uptake rates between experiments were probably caused by differences in algal morphology. The *Laminaria* tissue differed in thallus thickness and thus had different surface to volume ratios. Similarly to *L. digitata*, *L. saccharina* had its highest DIC uptake rate around 7.5, but this species reached slightly higher maximum rates, up to 460 μmol DIC kg FW^{-1} min^{-1} (Fig. 4).

AZ had an inhibitory effect on the carbon uptake rate in *L. digitata* from the start of the experiment. The effect comprised two components, one effect as a buffer and one as an inhibitor (Fig. 3b). The buffering effect of AZ on the pH drift was largest between pH 7.5 and 8.0 (around 35%), while the inhibitory effect of AZ increased from 21% to 55% between pH 7.0 to 8.5 (Table 3).

In *L. digitata* the inhibition by VAN was initially exclusively caused by its buffering effect (20–28% between pH 7.0 and 8.5; Table 3, Fig. 3c), but around pH 7.8 the non-buffering part of the inhibition appeared and at pH 8.5 it was twice that of the buffering effect. The pH at which the VAN inhibitory effect became apparent is most probably of no importance as it is the time necessary for VAN to penetrate into the cells, in combination with phosphate availability to the alga, which determines the response. The effect of phosphate availability on VAN inhibition was tested only for *L. saccharina*, and in this species the inhibitory effect of VAN was greatly enhanced when the algae had been starved for phosphate. When phosphate-enriched algae were treated with VAN there was a buffer effect during the entire experiment, while the non-buffering inhibitory effect of VAN was observed only from pH 9.15 and above (Table 3; Fig. 4a). In phosphate-starved algae there was a true inhibitory effect of VAN during the whole experiment of 20–23% (Table 3; Fig. 4b) even immediately after start. The alga probably does not distinguish between the two chemical analogues VAN and phosphate. The enhanced effect of VAN after phosphate depletion could be the result of either a faster transport of VAN into the cells due to the low external phosphate or a stronger competitive inhibition by VAN of H^+-ATPases inside the cells due to a lowered internal ATP-level.

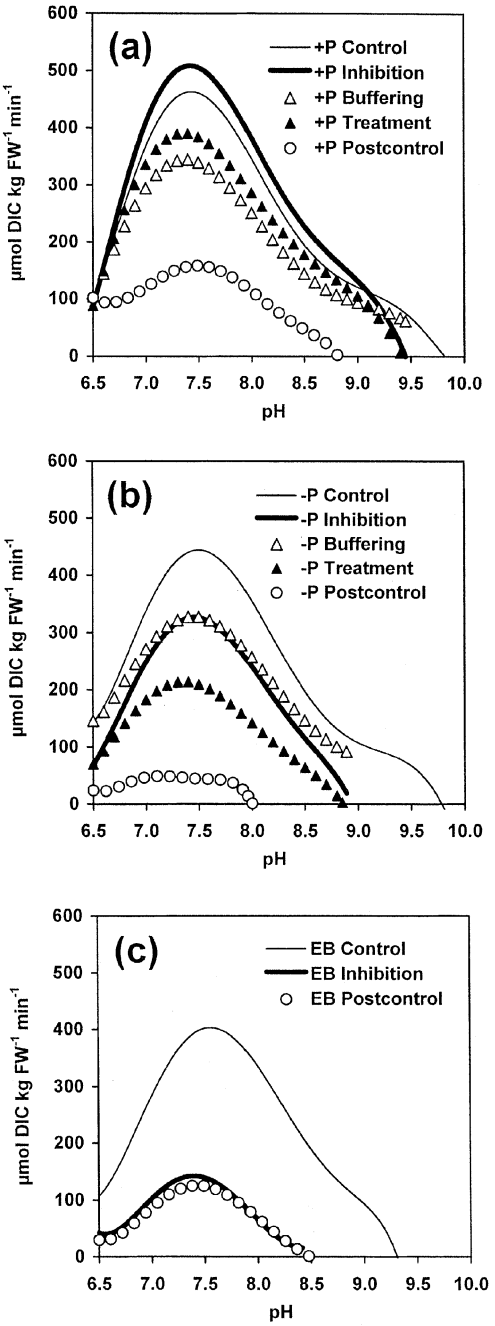

Figure 4. Results of the pH-drift experiments with *Laminaria saccharina* presented as DIC uptake rates calculated for each 0.01 pH unit between 6.5 and 10.0. Each experiment includes three measuring days for control (without inhibitor or buffer), treatment (with inhibitor or buffer) and postcontrol (after rinsing off the inhibitor or buffer), respectively. The treatment is also subdivided into a buffering effect and an inhibition effect. Treatments: (a) phosphate-enriched material treated with 0.2 mM VAN, (b) phosphate-starved material treated with 0.2 mM VAN, (c) normally cultivated material treated with 4 μM EB.

Table 3. Inhibition (−) or stimulation (+) of 0.2 mM AZ, 0.2 mM VAN, and 0.2 mM TRIS in *Laminaria digitata* and *L. saccharina*, expressed as % of the DIC uptake rate (μmol DIC kg FW^{-1} min^{-1}) of the control for each treatment at different pH. The treatment is also subdivided into a buffering effect and an inhibition effect. * = above pH compensation point. CV<2% for all values.

	pH	7.0	7.5	8.0	8.5	9.0
L. digitata	AZ Buffering	−26	−37	−34	−25	*
	AZ Inhibition	−21	−24	−41	−55	*
	AZ Treatment	−47	−61	−75	−80	*
	AZ Postcontrol	−17	−3	+12	+30	+53
L. digitata	VAN Buffering	−20	−26	−28	−26	*
	VAN Inhibition	+3	+3	−8	−46	*
	VAN Treatment	−17	−23	−36	−72	*
	VAN Postcontrol	−78	−83	−87	*	*
L. digitata	TRIS buffering	−13	−25	−30	−28	−20
	TRIS Inhibition	−8	+6	0	−7	−12
	TRIS Treatment	−21	−19	−29	−35	−32
	TRIS postcontrol	+1	+1	−4	0	+24
L. saccharina	VAN+P Buffering	−20	−26	−28	−26	−23
	VAN+P Inhibition	+11	+10	+10	+17	+9
	VAN+P Treatment	−8	−17	−17	−9	−13
	VAN+P Postcontrol	−67	−66	−67	−76	*
L. saccharina	VAN-P Buffering	−20	−26	−28	−26	*
	VAN-P Inhibition	−26	−27	−32	−42	*
	VAN-P Treatment	−46	−53	−60	−68	*
	VAN-P Postcontrol	−86	−90	−100	*	*

Treatment with 4 μM EB had a strong inhibitory effect on carbon uptake in both species, especially in *L. saccharina* where the inhibition was very strong already from the start of the experiment (Figs 3e, 4c). Addition of 0.2 mM TRIS had an inhibitory effect on the DIC uptake rate between pH 7.0 and 9.0 between 13 and 30% (Table 3, Fig. 3f), and this effect consisted practically entirely of the action of TRIS as a buffer. DIDS did not have a significant effect on the DIC uptake in *L. digitata* below pH 9.5 (Fig. 3d). Treatment with 1 mM MLD did not have any inhibitory effect on DIC uptake for either *L. digitata* or *L. saccharina*.

The postcontrol runs illustrate the long-term effects of the different inhibitors and TRIS buffer (Figs 3, 4). In the case of AZ and TRIS, the algae recovered after overnight incubation in new NSW and for AZ the postcontrol even showed a small stimulation of the DIC uptake rate from pH 8.0 (Fig. 3b). This shows that neither of these compounds has a long-term toxic effect on the *Laminaria* species when applied in low concentrations. For VAN and EB, the DIC uptake rates during the postcontrol runs were far lower than those of both controls and treatments. For DIDS this was slightly lower. These observations, again, indicate that VAN was still active inside the cell during the postcontrol run, and that EB and DIDS could not be washed away from the cell membrane by rinsing the algae with NSW. Also for the postcontrol DIC uptake rates, there was a clear difference between phosphate-enriched and phosphate-starved *L. saccharina*, the latter having a lower rate.

Discussion

Reference experiments

As CO_2 is consumed during photosynthesis in a closed system, it will continuously be replenished from the pool of HCO_3^- (and from CO_3^{2-}) in order to maintain the carbonate equilibrium in the seawater medium. Conversion of HCO_3^- into CO_2includes a consumption of protons and results in a pH increase of the

medium. This pH change can be used as a measure of photosynthesis as described by Axelsson (1988). The ability to raise the pH in a closed system above 9.0 is generally known as an indication that the alga is capable of using HCO_3^- for photosynthesis (Maberly, 1990). The *Laminaria* species used in this investigation can thus be considered as being HCO_3^- users, which is in accordance with previous work on these species (Axelsson & Uusitalo, 1988; Surif & Raven, 1989; Maberly, 1990; Haglund et al., 1992b; Larsson & Axelsson, 1999).

Alkalinity

Apart from photosynthesis, other processes can also affect the pH of the growth medium. Assimilation of e.g. NO_3^- or NH_4^+ generates OH^- ions and H^+, respectively. Alkalinity of seawater is defined as the amount of protons required to 'neutralise' 1 l of seawater. In practice, this is in seawater equivalent to the sum of the concentrations of the anions of carbonic and other weak acids and is associated with the charge balance. Removal from or addition to the medium of the neutral CO_2 during photosynthesis or respiration does not affect alkalinity, whereas uptake of the charged NO_3^- or NH_4^+ ions does (Brewer & Goldman, 1976). This was the rationale for undertaking the experiments where pH, alkalinity and total DIC were monitored simultaneously during photosynthesis. Alkalinity remained constant throughout the 12-h incubation, except with the addition of AZ where a very small increase was recorded, while simultaneously pH increased and total carbon decreased. A theoretical pH/DIC relationship for seawater, assuming a constant alkalinity, was calculated from the measured alkalinity and the experimental temperature and salinity conditions. These calculated values for pH and DIC were in agreement with the values obtained experimentally and we thus conclude that our observed pH changes in the pH-drift experiments were caused by the uptake of carbon from the medium.

Buffer capacity of inhibitors

Another obstacle in the evaluation of pH-drift experiments is the interference of other buffers than the carbonate system on the pH drift. We therefore measured the buffering capacity of NSW with addition of all inhibitors used in experiments. The relative change in buffering capacity with addition of inhibitor was then used to correct the calculated DIC uptake rates for the effect of the inhibitors as buffers. It appeared that a large part of the inhibitions by both AZ and VAN were in fact a buffer effect. We confirmed the validity of these corrections by using a low concentration of a proton buffer (TRIS) with a pK_a value and buffer capacity similar to VAN. The discovery of the large buffering capacity of AZ in oceanic seawater (this study) as well as in brackish seawater (Snoeijs et al., 2002; Ray et al. 2003) will have consequences for the interpretation of earlier experimental studies using AZ as an inhibitor (e.g. Andría et al., 1999; Granbom & Pedersén, 1999; Larsson & Axelsson, 1999; Axelsson et al., 2000).

External conversion of HCO_3^- to CO_2 and anion exchange

From our pH-drift experiments we conclude that AZ has an inhibitory effect on carbon uptake rates in *L. digitata*. This effect was completely reversed by rinsing and overnight incubation in new seawater, suggesting that AZ acts on the outside of the plasma membrane as suggested previously by Moroney et al. (1985). These results confirm previous studies, which have shown that photosynthesis in *Laminaria* can be inhibited with AZ (Haglund et al., 1992b; Larsson & Axelsson, 1999). The presence of an anion exchange protein for direct uptake of HCO_3^- has previously been demonstrated in a number of macroalgal species using the inhibitor DIDS (Drechsler et al., 1993; Andría et al., 1999; Granbom & Pedersén, 1999; Larsson & Axelsson, 1999), but the two *Laminaria* species used in the present study have been reported to be insensitive to this inhibitor (Larsson & Axelsson, 1999). In the present study, when *L. digitata* was treated with DIDS in pH-drift experiments, there was a small effect above pH 9.5 with a reduction of the pH compensation point by 0.2 pH units compared to the control. From our results we conclude that *L. digitata* has a direct uptake of HCO_3^-, but only at extreme, high pH. This mechanism is probably of negligible importance in the normal carbon uptake of this species, but may be activated in extreme situations as a survival mechanism.

Plasma membrane proton pumps

From the inhibitory effect of VAN and EB on photosynthetic DIC uptake, we conclude that a P-type H^+-ATPase is involved in carbon acquisition in both investigated *Laminaria* species. VAN is the most well known and widely used inhibitor of plasma membrane P-type H^+-ATPases in plants (Michelet & Boutry, 1995) and algae (Palmqvist et al., 1988; Karlsson

et al., 1994). The action of this inhibitor is reportedly directly against the function of the H^+-ATPase in the plasma membrane and thus inhibits cellular H^+ extrusion (Beffagna & Romani, 1988; Marré et al., 1988). However, VAN is not fully specific for the plasma membrane P-type H^+-ATPase and that is why we also checked phosphatase inhibition with the help of MLD. VAN is a phosphate analogue that competes with the phosphate from ATP for the aspartic acid phosphorylation site on the enzyme (Briskin & Hanson, 1992). Gilmour et al. (1985) showed that phosphate starvation enhances the inhibition of photosynthesis by VAN in *Dunaliella parva*, which is consistent with our observation that the inhibition of DIC uptake by VAN in *L. saccharina* is much stronger in algae that had been starved for phosphate compared to algae that had been cultivated in phosphate-enriched medium. The transport mechanism of VAN into the cell to the phosphorylation site on the inside of the cell membrane is probably the same as that of PO_4^{3-} uptake, which is known to occur both as symport with H^+ in plants (Taiz & Zeiger, 1998) and via a DIDS-sensitive anion exchange protein in mammalian red blood cells (Cabantchik & Greger, 1992; Garner et al., 1997).

Active carbon uptake

Lucas (1975) showed that *Chara corallina* uses both CO_2 and HCO_3^- for photosynthesis and that HCO_3^- assimilation is inhibited by pH buffers. Based on the assumption that HCO_3^--supported photosynthesis proceeds faster than the rate of conversion of HCO_3^- to CO_2, he suggested that HCO_3^- is transported across the plasma membrane and that the buffers act as competitive inhibitors at the HCO_3^- binding site of the transport proteins. In contrast, Walker et al. (1980) pointed out that the pH close to the cell surface, in the acid bands which *C. corallina* develops during photosynthesis, might be sufficiently low to allow a higher rate of HCO_3^- to CO_2 conversion. They argued in favour of CO_2 as the carbon species being transported across the plasma membrane. Price & Badger (1985) suggested that the strong inhibitory effect of pH buffers on photosynthesis in *Chara* is caused by dissipation of a pH gradient between the bulk medium and the periplasmic space. The observations of Mimura et al. (1993) that photosynthesis in *C. corallina* at both low (5.5) and high (8.5) pH is sensitive to VAN and internal ATP depletion made them conclude that assimilation of both CO_2 and HCO_3^- depends on the activity of H^+-ATPases in the plasma membrane. In *L. digitata*, the mechanism by which the H^+-ATPases supports DIC uptake could be either a H^+/HCO_3^- co-transport or a low-pH-enhanced conversion of HCO_3^- to CO_2 in the periplasmic space. The observation that inhibition by AZ increases with increasing pH indicates that the DIC uptake is dependent on an external CA even when the ambient pH no longer promotes the conversion of HCO_3^- to CO_2. In this case, the external CA would operate in zones of lower-than-ambient pH, possibly in the direct vicinity of the H^+-extruding ATPases. Furthermore, the evidence from many marine photosynthetic organisms suggests that the H^+-electrochemical potential gradient across the plasma membrane is very small (a few kJ per mole H^+, inside negative; Raven, 1997) and probably insufficient to energise a H^+/HCO_3^- co-transport. This would also support that CO_2 is the carbon species that is transported across the plasma membrane in *Laminaria*.

In the present study, we show that the alkalinity of the seawater does not decrease during pH-drift with *Laminaria* in a closed system, and proton extrusion to the medium by ATPases must therefore be balanced in some way, e.g. by H^+ uptake or OH^- extrusion. Simultaneous extrusion of H^+ and OH^- has been observed in e.g. *Chara*, where alternating acid and alkaline bands develop along the outer cell wall and in *Elodea* with H^+ extrusion on the lower side of the leaf and OH^- extrusion on the upper side (Lucas, 1983). A prerequisite for such mechanisms to be efficient is distinct spatial separation of H^+ and OH^- extrusion to avoid futile cycling of ions across the membrane. This is possible in *Chara* with its giant cells and in leaves of *Elodea*, which are differentiated in an upper and a lower side. Such a spatial separation is problematic in the small cells of *Laminaria*, and a polarity of the blade and/or a differentiation between cells concerning H^+/OH^- extrusion has not been reported in this genus. Further studies are necessary to resolve how *Laminaria* species maintain their internal charge balance during active carbon uptake.

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Growth and C:N:P ratios in copepods grazing on N- or Si-limited phytoplankton blooms

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Abstract

The aim of this study was to investigate how nutrient limitation in phytoplankton blooms affects growth and C:N:P ratios in marine pelagic copepods. We performed two mesocosm experiments on the Atlantic coast near Trondhjem (Norway). Si-limitation in a phytoplankton bloom was triggered by N and P additions (NP treatment) and N-limitation was triggered by N, P and Si additions (NPSi treatment). Both nutrient treatments stimulated microalgal growth and increased the biomass of the phytoplankton manifold. The initial phytoplankton community consisted of flagellates and diatoms. Throughout both experiments, community composition stayed relatively stable and diverse in the NP treatments, but in the NPSi treatments large and heavily silicified diatoms came to dominate completely. Phytoplankton C:N ratios in the Si-limited blooms were close to the Redfield ratio of 6.6 (on a molar basis), but they were higher in the Control treatment without nutrient additions (ca. 8.6) and up to 14 in heavily N-limited blooms. When phytoplankton blooms (chlorophyll $a > 25$ nmol l^{-1}) had established, wild copepods were added to the mesocosms. During Si-limitation the copepod density increased by ca. 40% in one of the experiments, while the C:N ratio was 5.5–6 in the copepods. During N-limitation, the copepod density stayed stable, while the C:N ratio increased to ca. 7 in the course of the experiment. In the other experiment the copepod density decreased by ca. 25%, irrespective of nutrient treatment (C:N ratio ca. 9). The N:P ratios in the copepods varied between 16 and 22 and were not different in the NP and NPSi treatments. Our study shows that N-limitation in phytoplankton cells can increase the C:N ratio of their grazers, which has a reportedly negative effect on copepod growth and reproduction. Our study also shows that copepod populations can be regulated by seawater Si:N ratios via diatoms: at high ratios the growth from eggs/nauplii to copepodites was hampered. High Si:N ratios provide diatoms with possibilities to escape from grazing (large species, heavy silicification, excretion of secondary metabolites), leading to the accumulation of algal biomass without transfer to higher trophic levels.

Introduction

Fundamental changes in phytoplankton community composition and biomass as a consequence of eutrophication have been reported for many aquatic systems world-wide (Smith et al., 1999). Such changes are expected to have profound bottom-up effects on trophic relationships, which may go beyond the phyto- and zooplankton levels and affect higher trophic levels such as fish. In the present study we focus on effects of inorganic nutrients on trophic interactions between phytoplankton and pelagic copepods (crustaceans), which are major grazers in the sea and an important link to fish. In marine ecosystems, nitrogen is usually the limiting macronutrient for algal growth (Caraco et al., 1990). However, with eutrophication silicate limitation may occur instead (Conley et al., 1993), shifting phytoplankton community composition from diatom-dominated to flagellate-dominated (Officer & Ryther, 1980; Smayda, 1990). It is expected that zooplankton grazers will be affected when their microalgal prey changes in abundance, species

composition, chemical composition and palatability by eutrophication.

The ratios of C, N and P are of paramount importance for production, nutrient cycling and food-web dynamics across Earth's diverse habitats (Elser et al., 2000). Although the Redfield ratio of C:N:P = 106:16:1 (Redfield et al., 1963) is often encountered in algae, their elemental composition is generally very plastic in nature (Geider & La Roche, 2002). Animals regulate their elemental content more strictly through prey selection and physiological mechanisms (Sterner & Hessen, 1994). Previous studies have suggested that both freshwater and marine copepod grazers can be growth-limited by a C:N ratio of ingested food that is too high (Hessen, 1992). Also copepod egg production has been found to be negatively correlated to algal C:N ratios (Kiørboe, 1989). A strong mismatch in ratios between the requirements of a grazer and its diet should affect grazer growth and reproduction.

In the present study we used mesocosm experiments to assess how different phytoplankton blooms (N- or Si-limited) will affect growth and C:N:P ratios in pelagic copepods. Mesocosms have frequently been used for studying the effects of nutrient dynamics on phytoplankton growth and taxonomic composition (e.g. Egge & Jacobsen, 1997; Carlsson & Granéli, 1999) and for studying food web interactions (e.g. Prins et al., 1999; Hansen et al., 2000). Detailed grazing studies have mainly been conducted under more controlled conditions, e.g. by feeding algal blooms grown in mesocosms to single copepod species (Turner et al., 1999; Nejstgaard et al., 2001) or to wild copepod assemblages (Andersson et al., 2003) in small incubations. In our experiments, we combined nutrient limitation of phytoplankton and zooplankton grazing into one experimental set-up. First different phytoplankton communities were created by nutrient manipulations and then natural copepod assemblages were added to graze upon these communities. Our aim was to keep the complex structure of the pelagic food web as natural as possible, but top-down effects (copepod predators) were removed to be able to focus on bottom-up effects on the copepods.

Material and methods

Field sites and experimental design

Two mesocosm experiments were performed on the Norwegian Atlantic coast in summer 1999. The first experiment (Exp 1, Day 1–9) was carried out from 12 to 20 August in an outdoor basin at the Trondhjem Biological Station, Trondhjem University, ca. 120 km inside the Trondhjem fjord (63° 36′ N, 10° 23′ E). The second experiment (Exp 2, Day 1–12) was carried out from 29 August to 9 September at the Sletvik Biological Field Station, Trondhjem University, in a natural lagoon (Hopavågen) close to the mouth of the Trondhjem fjord (63° 40′ N, 09° 39′ E).

The mesocosms consisted of white polyethylene enclosures with a diameter of 1.0 m, a water-filled depth of 2.5 m and a volume of ca. 2000 l. Nine mesocosms in Exp 1 and six mesocosms in Exp 2 were, with random placement, submerged into the water and attached to rubber rings on a floating raft. Natural seawater was pumped into the mesocosms through a 90 μm-mesh plankton net, thus excluding larger zooplankton. The nutrient enrichment treatments followed the Redfield ratio for N:P (16:1) and three times the by Redfield mentioned optimal ratio for diatom growth Si:N (3:1) to ensure excess Si (ratios given on a molar basis; Redfield et al., 1963). Every morning (after sampling), 4 μM $NaNO_3$ and 0.25 μM NaH_2PO_4 were added to the NP treatment and 12 μM Na_2SiO_3, 4 μM $NaNO_3$ and 0.25 μM NaH_2PO_4 to the NPSi treatment. In Exp 1 also a Control treatment (without N, P or Si additions) was included. All treatments were conducted in triplicate. Carbon, in the form of 28 μM $NaHCO_3$ (cf. Redfield ratio C:N:P = 106:16:1), as well as trace metals, chelators and vitamins [in 0.5% of f/2 medium concentrations (Guillard & Ryther 1962), but excluding thiamine], were added to all mesocosms daily. In Exp 2 no more nutrients were added on Days 9–12. Wild zooplankton dominated by copepods was collected in the morning with a 200 μm-mesh plankton net at 0–35 m depth in the sea and starved for ca. 4 h in an aquarium. Within each experiment the same zooplankton inoculate was added to all mesocosms on Day 6 at noon. The copepod density (mean ± SD) of the inoculate was 11.9 ± 1.5 ind l^{-1} in Exp 1 and 18.5 ± 2.7 ind l^{-1} in Exp 2.

Sampling and field measurements

In the morning of each experimental day the mesocosms were stirred, after which 10 l of water were sampled from each of the mesocosms and filtered through a 200 μm-mesh plankton net. Twenty-ml water samples were stored at −20 °C in scintillation vials for later nutrient analyses. For chlorophyll *a* (chl *a*) analysis, 100- to 500-ml water samples were filtered

on GF/F Whatman™filters. Chl *a* was extracted in methanol and its concentration was determined spectrophotometrically according to the Norwegian Standard (NS 4767). For later C, N and P analyses, 100- to 500-ml water samples were filtered on Whatman™ GF/F filters, immediately frozen in liquid nitrogen and stored at −86 °C until analysis.

Copepod samples were taken daily from each mesocosm on Days 6-9 of Exp 1 and on Days 6–12 of Exp 2 by pouring 8 to 15 l of water through a 200 μm-mesh plankton net. The water was immediately poured back into its mesocosm. The fraction >200 μm left on the net was rinsed with filtered seawater and collected on a Whatman™ GF/C filter for later chl *a*, C, N and P analyses. The filters were immediately frozen in liquid nitrogen and stored at −86 °C until analysis.

Every second day, one 100 ml phytoplankton sample and one copepod sample (concentrated in 100 ml from 8–15 l) from each mesocosm were preserved with acid Lugol's solution for taxonomic analyses. Salinity, water temperature and pH in the mesocosms were measured daily at noon. Light measurements (PAR) were obtained from continuous recordings available at the biological stations. The mesocosms were stirred three times each day (at 8:00, 13:00 and 19:00 h).

Taxonomic analyses

Phytoplankton 2–200 μm sampled on Days 1, 3, 5, 7, 9 and 11 was identified and counted in 10 or 25 ml counting chambers using the Utermöhl technique (Utermöhl, 1931) at ×100 and ×400 magnification under an inverted light microscope (Olympus™ IMT). At least 400 cells were counted in each sample. The algae were subdivided into six groups: diatoms, prymnesiophytes + chrysophytes (in all samples >90% prymnesiophytes), chlorophytes + prasinophytes, cryptophytes and dinoflagellates according to Tomas (1997) and a rest group named 'other phytoplankton taxa'. Phytoplankton biovolumes were calculated according to Hillebrand et al. (1999) based on at least 25 measurements for species >10 μm and at least 10 measurements for species <10 μm (Snoeijs et al., 2002). Mesozooplankton >200 μm was counted under a binocular microscope (Wild™ M5-26590) at ×50 magnification. Copepod identification to genus and calculation of biovolume was performed according to Mauchline (1998). The density of copepods was corrected for the sampled water volume since the fraction <200 μm of the water from which the zooplankton samples were taken was poured back into the mesocosms.

Chemical analyses

Water nutrient concentrations, dissolved inorganic nitrogen (DIN = NO_3-N + NO_2-N + NH_4-N), dissolved inorganic phosphorus (DIP = PO_4-P) and dissolved silicate (SiO_2-Si) were analysed with a Skalar San Plus System according to methods described in Grasshoff (1999). Total C and N (Exp 1) or C, N and P (Exp 2) on freeze-dried phytoplankton and copepod filters were analysed with a LECO CHNS-932 elemental analyser. Chl *a* on the copepod filters was extracted and analysed by HPLC according to methods modified after Wright et al. (1997) and Wright & Jeffrey (1997) and described in detail by Andersson et al. (2003). With the help of these chl *a* values and known C:chl *a*, N:chl *a* and P:chl *a* ratios of the phytoplankton fraction, the C, N and P values for the copepods were corrected for the occurrence of phytoplankton cells in the copepod fraction.

Statistics

Samples were not independent because they were taken from the same mesocosms on different days. Therefore, comparisons of means were carried out by paired *t*-tests or by repeated measures ANOVA (rm ANOVA) with time as repeated measures factor. Statistics were performed with the programme STATISTICA™ , Version 6.0. Significance was accepted at $p < 0.05$ throughout this paper.

Results

Abiotic factors

Water temperature and irradiance at the water surface were slightly higher in Exp 1 than in Exp 2 (mean temperature ± SD: 13.3 ± 0.7 and 12.6 ± 0.4 °C, respectively; daily mean irradiance 370 and 324 μmol photons PAR m^{-2} s^{-1}, respectively). In the fertilised mesocosms the pH increased as a result of photosynthetic carbon uptake during the experiments and it was always higher in the NPSi treatment than in the NP treatment. The pH (mean ± SD) at the end of the experiments was in Exp 1 (starting from pH 8.47): 8.40 ± 0.02 (Control), 8.72 ± 0.04 (NP) and 9.23 ± 0.08 (NPSi), and in Exp 2 (starting from pH 8.15): 8.45 ± 0.01 (NP) and 9.02 ± 0.03 (NPSi). Salinity (mean ±

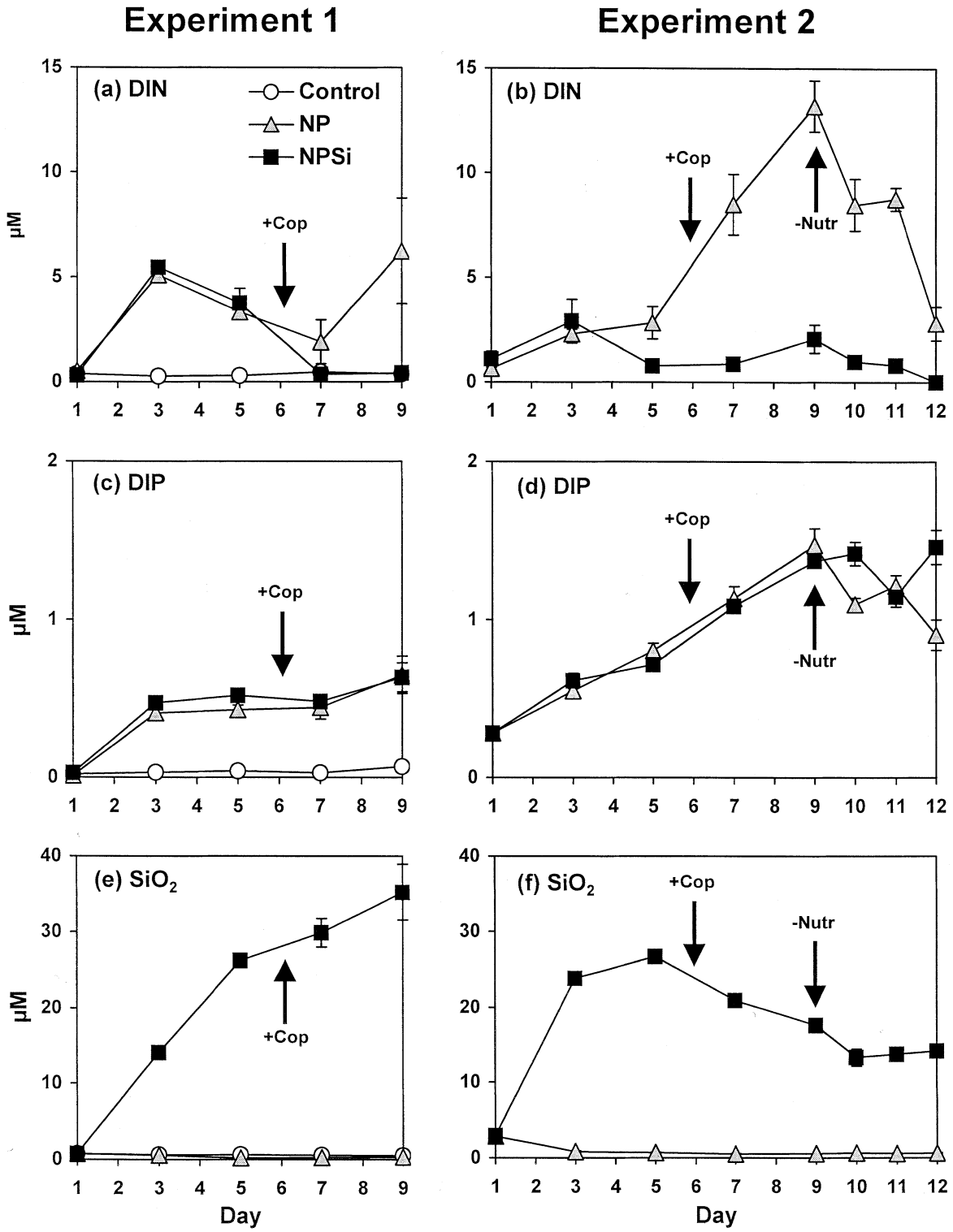

Figure 1. Mean water nutrient concentrations ($n = 3$) during the two mesocosm experiments: (a, b) DIN (NO_3-N + NO_2-N + NH_4-N), (c, d) DIP (PO_4-P) and (e, f) silicate (SiO_2-Si). Arrows indicate the days of copepod additions (+Cop) and the termination of nutrient additions (−Nutr). Error bars represent 1 standard error of the mean.

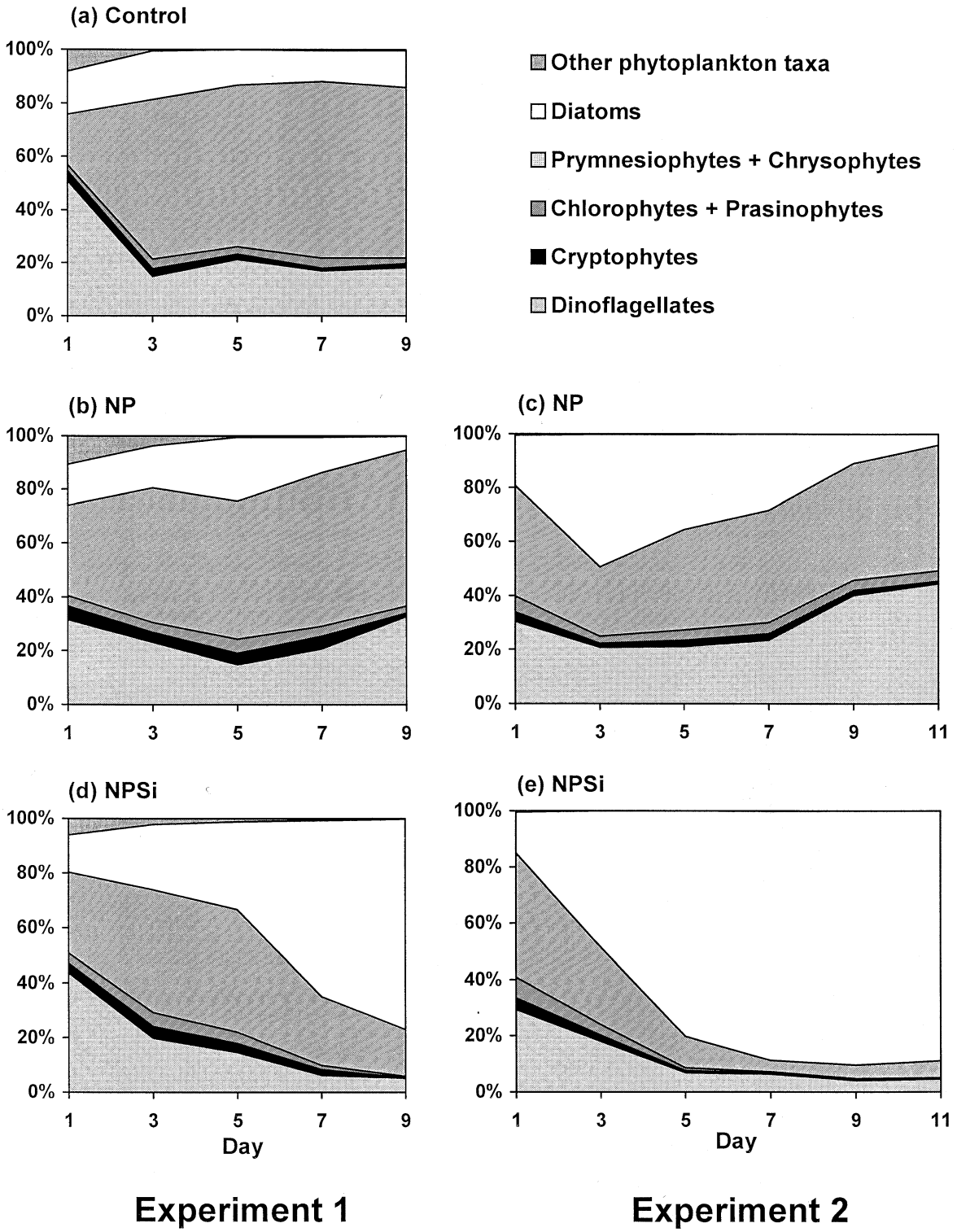

Figure 2. Development of quantitative phytoplankton community composition ($n = 3$) during the two mesocosm experiments: (a) Control treatment without nutrient additions, (b, c) NP treatments, (d, e) NPSi treatments.

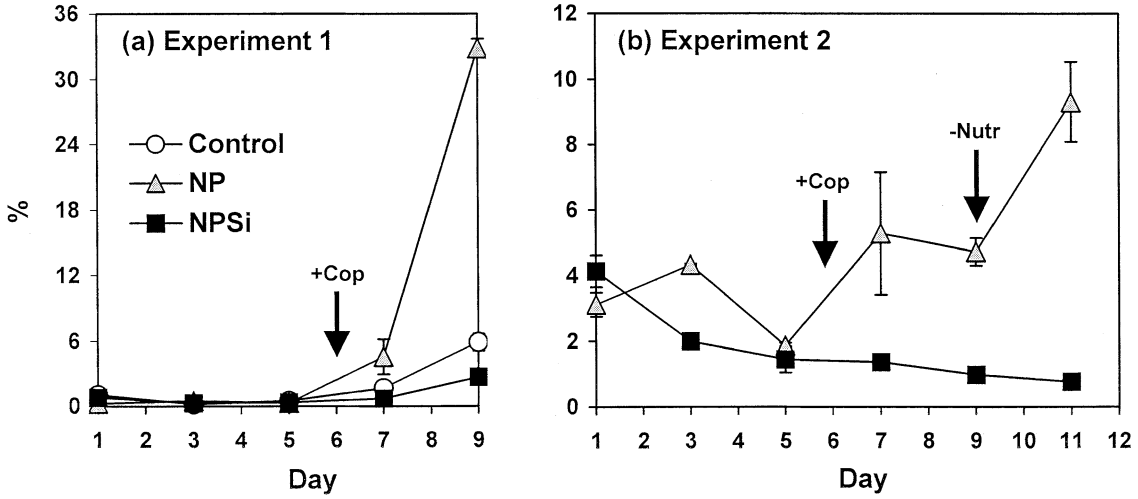

Figure 3. Relative abundance of heterotrophic cells <200 μm in the phytoplankton fraction ($n = 3$) during the two mesocosm experiments. Arrows indicate the days of copepod additions (+Cop) and the termination of nutrient additions (−Nutr). Error bars represent 1 standard error of the mean.

SD) was stable throughout the experiments, 26.5 ± 0.4 practical salinity units (PSU) in Exp 1 and 30.1 ± 0.3 PSU in Exp 2.

Nutrient concentrations

In the NPSi treatments, DIN in the seawater was below 1 μM after 6 days in Exp 1 and after 4 days in Exp 2 (Figs 1a,b). From Day 5 on, the phytoplankton in the NPSi treatment of Exp 2 consumed more silicate than the daily dose added and the concentrations in the water decreased, but silicate never became limiting (Figs 1g,h). In the NP treatments, silicate was depleted already after 2 days in both experiments. In the Control treatment of Exp 1 water nutrient concentrations were low throughout the experiment. DIP was never limiting algal growth in any of the NP and NPSi treatments (Figs 1e,f). NH_4-N slightly increased in the NP treatment after Day 5 in Exp 2 (up to 1–2 μM), but stayed low in Exp 1. After nutrient additions were stopped on Day 9 of Exp 2, DIN and DIP decreased in the NP treatment, while in the NPSi treatment DIP ceased to increase. No changes in water nutrient concentrations affecting nutrient limitation conditions occurred from Day 9 to Day 12 as a result of the termination of nutrient additions on Day 9.

Taxonomic composition of the phytoplankton

At the level of the major algal groups, the initial community composition was similar in the two experiments with diatoms, prymnesiophytes + chrysophytes and dinoflagellates as the numerically most abundant groups (Fig. 2). However, dinoflagellates dominated in terms of biomass because several large species were present, especially in Exp 2. With time, the nutrient treatments significantly altered species composition. In the Control treatment, prymnesiophytes + chrysophytes became more abundant while the relative abundances of diatoms and dinoflagellates decreased (Fig. 2a). Prymnesiophytes + chrysophytes also increased in the NP treatment, and at the end of the experiments they had increased and the relative abundance of diatoms had decreased, similar to the Control treatment (Figs 2b,c). However, in both experiments an initial relative increase in diatoms was observed in the NP treatments, whereas prymnesiophytes + chrysophytes and dinoflagellates increased and dominated towards the end of the experiments after total Si-depletion. In the NPSi treatments (Figs 2d,e), diatoms became the sole dominant group after 3 to 5 days and continued to dominate until the end of the experiments.

Diatom species composition was similar in both experiments. The dominating species of diatoms were *Chaetoceros* spp., *Cylindrotheca closterium/Nitzschia longissima*, *Guinardia* sp., *Leptocylindrus* sp., *Pseudonitzschia* spp., *Skeletonema* sp. and *Thalassiosira* spp. In the course of the experiments the diatoms in the NPSi treatment became more heavily silicified (microscopic observations) and large diatom species became more abundant compared to the Control and NP treatments. Large *Ceratium* species, mainly *C. fusus*

and *C. lineatum*, dominated the dinoflagellates, but also *Scrippsiella*, *Protoperidinium*, *Gymnodinium* and *Gyrodinium* species were abundant.

Heterotrophic cells in the phytoplankton fraction consisted mainly of microzooplankton (*Lohmaniella* sp. and *Strombidium* sp. and tintinnids), while some copepod nauplii were recorded as well. Towards the end of both experiments, the relative abundances of heterotrophic cells in the NP treatments increased, but not in the NPSi treatments (Fig. 3). In Exp 1 absolute heterotrophic cell numbers were 4× and 14× higher in the NP treatment than in the NPSi and Control treatments, respectively, but in Exp 2 absolute heterotrophic cell numbers were only 1.7× higher in the NP treatment than in the NPSi treatment.

Net changes in phytoplankton community composition as a result of nutrient additions before copepods were added were expressed as the difference between Day 1 and Day 5 for each of the major phytoplankton groups (Fig. 4, indicated by N). In Exp 1, all groups increased more in abundance in the nutrient treatments than in the Control treatment. In both experiments, diatoms <20 μm (*Skeletonema*, *Chaetoceros*) increased more in the NPSi treatments than in the NP treatments (Fig. 4a). In Exp 2, diatoms >20 μm (*Thalassiosira*, *Pseudonitzschia*, *Leptocylindrus*, *Guinardia*) increased more (Fig. 4b) and prymnesiophytes + chrysophytes less (Fig. 4e) in the NPSi treatments than in the NP treatments. Trends were also observed for some other groups, but for these the differences between the treatments were not significant (paired *t*-tests).

Net changes in phytoplankton community composition as a combined result of nutrient and copepod additions were expressed as differences between Day 5 and Day 9 for the major algal groups (Fig. 4, indicated by NC). All phytoplankton groups decreased more in abundance than in the Control of Exp 1, except for dinoflagellates in Exp 1 and diatoms in Exp 2. In both experiments, diatoms >20 μm (*Thalassiosira*, *Pseudonitzschia*, *Leptocylindrus*, *Guinardia*) decreased in the NP treatments and increased in the NPSi treatments and the differences between the treatments were significant (paired *t*-tests; Fig. 4b). This was also found for diatoms <20 μm (*Skeletonema*, *Chaetoceros*), but there was only a significant difference between the treatments in Exp 1 (Fig. 4a). Trends were also observed for some other groups, but for these the differences between the treatments were not significant (paired *t*-tests). As changes in community composition were caused by two major constraints simultaneously (nutrients and grazing), it is not possible to distinguish the individual causal factors for each constraint separately.

Taxonomic composition of the copepods

Copepods dominated the zooplankton numbers in the 'copepod samples' with mean relative abundances of 76% in Exp 1 and 69% in Exp 2. On the basis of biovolume, this was 93% and 95%, respectively. The rest of the samples consisted mainly of the cladocerans *Podon* sp., *Evadne* sp. and *Bosmina* sp. Copepod taxonomic composition did not differ between the treatments within an experiment, but it did differ between the two experiments (Fig. 5). *Acartia* spp. and *Temora* sp. dominated in Exp 1 with relative abundances of ca. 50% each, whereas *Centropages* sp., *Oithona* sp. and *Pseudo/Paracalanus* spp. occurred in low numbers (Figs 5a,b,d). In Exp 2 (Figs 5c,e), the five genera were distributed more evenly and the assemblage was slightly more dynamic with time through small increases in the relative abundances of *Centropages* sp. and *Pseudo/Paracalanus* spp.

Phytoplankton biomass

Phytoplankton biomass immediately responded to the nutrient additions (Fig. 6). Maximum autotrophic phytoplankton cell numbers reached in Exp 1 were 25×10^6 cells l^{-1} (Control), 60×10^6 cells l^{-1} (NP treatment), 136×10^6 cells l^{-1} (NPSi treatment) and in Exp 2 these were 107×10^6 cells l^{-1} (NP treatment) and 408×10^6 cells l^{-1} (NPSi treatment). In the fertilised mesocosms large diatoms and dinoflagellates increased from Day 1 to Day 5 (Figs 4b, d) and thus biomass increased relatively more by the nutrient additions than cell numbers. Chl *a*, C and N in the fertilised phytoplankton were significantly higher than in the Control treatment already 48 hours after the start of Exp 1 (paired *t*-tests, Figs 6a,c,e). On Day 5 mean chl *a* concentrations in the nutrient treatments of both experiments were above 25 nmol l^{-1}, but still below 3 nmol l^{-1} in the Control treatment of Exp 1. After Day 5 the NPSi treatment always had higher phytoplankton biomass than the NP treatment and showed a different temporal pattern. Chl *a* concentrations ceased to increase in the NP treatment when the copepods were added whereas in the NPSi treatment chl *a* concentrations kept increasing until Day 9 (the day nutrient additions were stopped). C and N concentrations increased in concert with chl *a*, but

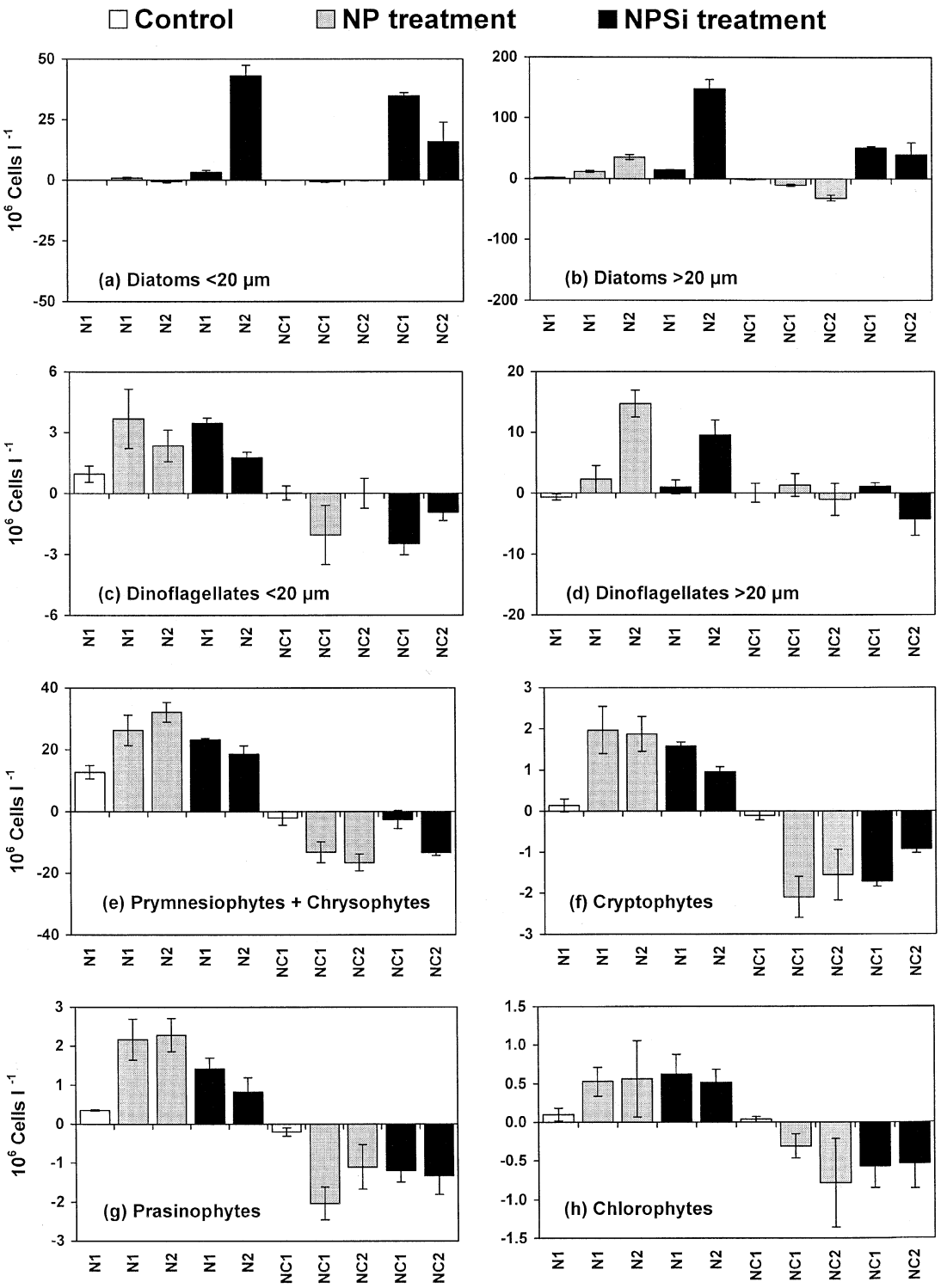

Figure 4. Changes in phytoplankton community composition as a result of nutrient addition (N) between Day 1 and Day 5 (the five left bars in each diagram) and changes in community composition as a combined result of nutrient and copepod additions (NC) between Day 5 and Day 9 (the five right bars in each diagram). Data from both experiments are included (N1, NC1 = Experiment 1; N2, NC2 = Experiment 2). Error bars represent 1 standard error of the mean.

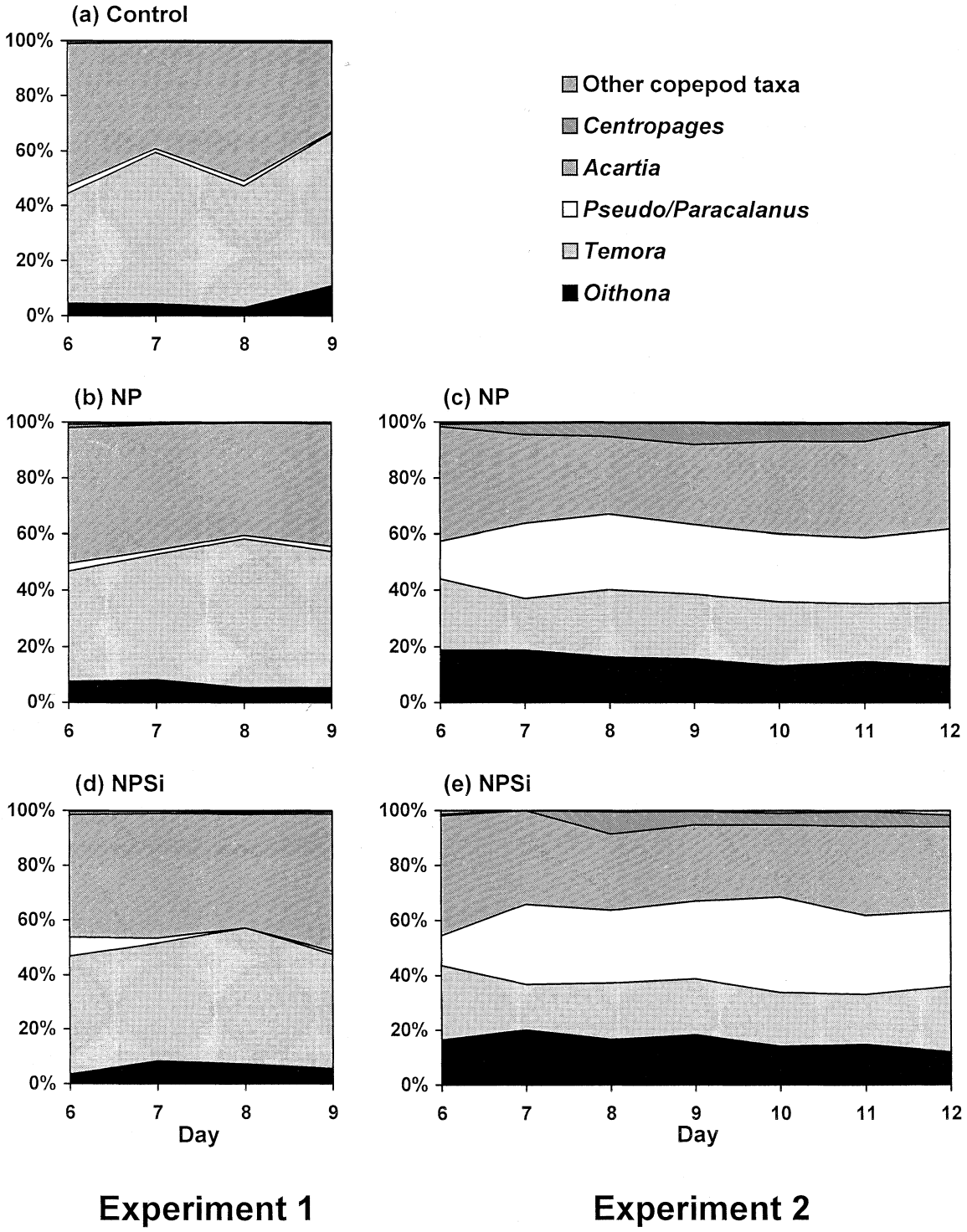

Figure 5. Development of copepod assemblage composition ($n = 3$) during the two mesocosm experiments: (a) Control treatment without nutrient additions, (b, c) NP treatments, (d, e) NPSi treatments.

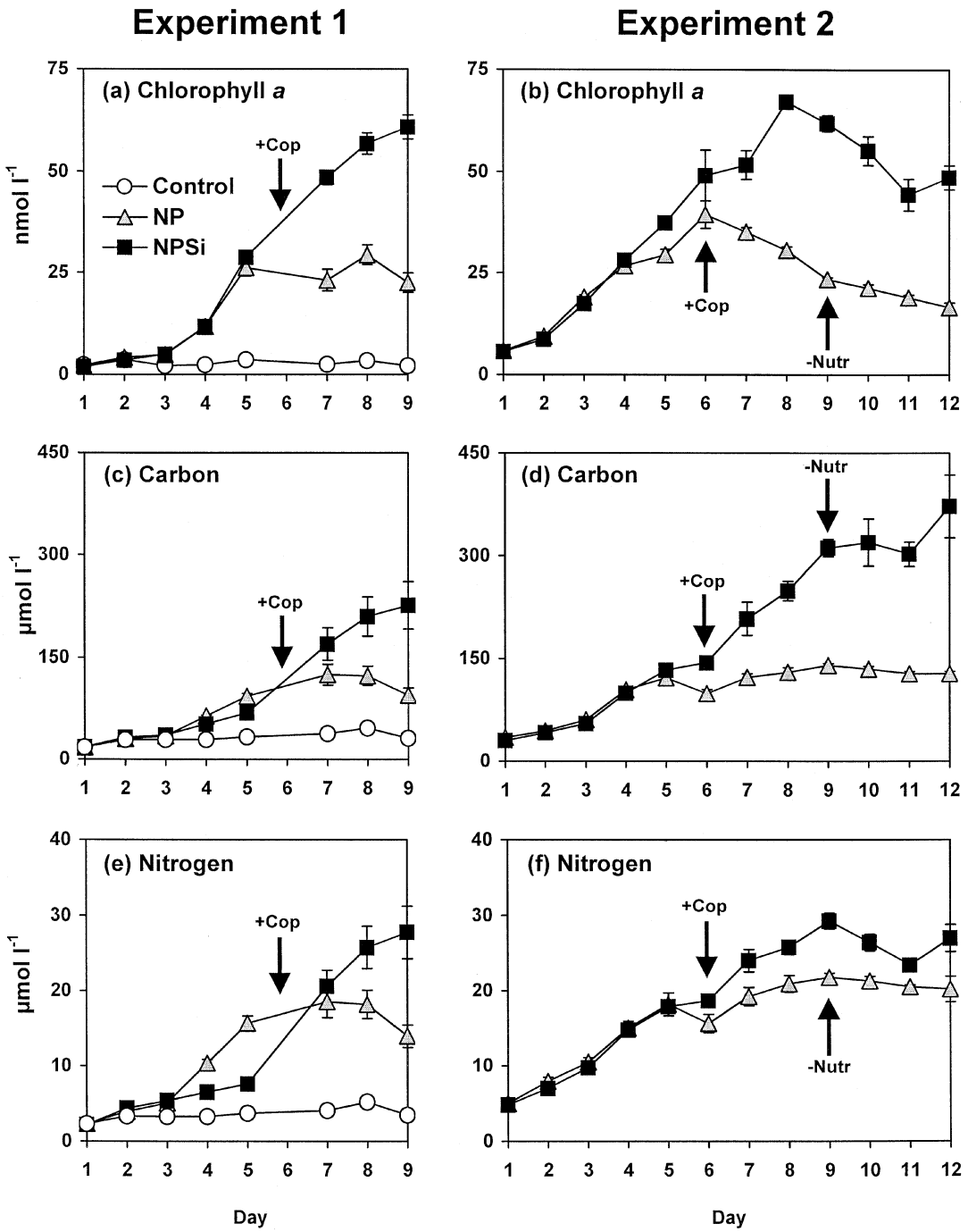

Figure 6. Phytoplankton biomass and nutrient content ($n = 3$) during the two mesocosm experiments, expressed as chlorophyll *a* concentration (a, b), carbon content (c, d) and nitrogen content (e, f). Arrows indicate the days of copepod additions (+Cop) and the termination of nutrient additions (−Nutr). Error bars represent 1 standard error of the mean.

when chl *a* decreased in Exp 2 (Fig. 6b), C did not follow (Fig. 6d), which was probably a result of a higher proportion of senescent cells and detritus after bloom 'crash'. In the N-limited NPSi treatment of Exp 2, N bound in the phytoplankton was lower relative to chl *a* than in the NP treatment (Fig. 6f). The phytoplankton P content (only analysed for Exp 2, not shown) showed a pattern similar to that of N.

Copepod density and biomass

The copepod density in the mesocosms developed in the same way in the three treatments of Exp 1 and decreased from ca. 12 down to ca. 9 ind l^{-1} during the three days after copepod addition (Fig. 7). In Exp 2 copepod density developed differently with time in the two treatments (rm ANOVA). Following an initial decrease from 19 to 16 ind l^{-1}, the number of copepods in the NPSi treatments stayed around 16 ind l^{-1} from Day 7 to Day 12 (Fig. 7b). Contrarily, in the NP treatment there was an increase between Day 6 and Day 12 from 18 to 25 ind l^{-1}. This implies that the number of copepods had increased through growth of eggs/nauplii $<90\,\mu m$ into copepodites $>200\,\mu m$ since Day 1 in the NP treatment of Exp 2.

In Exp 2 the nutrient contents of the copepods showed significant changes with time but not with treatment (Fig. 7d; rm ANOVA). The mean C content per individual almost doubled; it increased from 312 to 591 nmol between Day 6 and Day 12. Simultaneously, mean N and P content per individual increased from 57 to 89 nmol and from 3.4 to 4.6 nmol, respectively (Fig. 7f, data for P not shown). Also in Exp 1 the C and N contents per copepod individual tended to increase with time (Figs 7c,e). In the NPSi treatments the C, N and P contents increased significantly between Day 6 and Day 9 when nutrients were still added (rm ANOVA), but in the NP treatments it stayed the same. This was revised between Day 9 and Day 12 (Exp 2 only) when no more nutrients were added: nutrient contents per individual stayed constant in the NPSi treatment and increased in the NP treatment (rm ANOVA).

C:N and N:P ratios

The initial C:N ratio (mean $\pm$ SD) in the phytoplankton on Day 1 was 7.8 ± 0.5 in Exp 1 and 6.5 ± 0.5 in Exp 2. In the Control treatment of Exp 1 this ratio increased to ca. 8.8 (Fig. 8a). In the fertilised treatments the C:N ratio initially dropped before it increased to ca. 6.5 in the NP treatment and to ca. 8.5 in the NPSi treatment. In Exp 2 there was a similar initial drop in C:N ratio in both treatments before it increased to ca. 6.3 and stayed constant on this level in the NP treatment, but in the NPSi treatment it increased up to 13.7 (Fig. 8b). Thus, the C:N ratio of the Si-limited phytoplankton in the NP treatment was close to the Redfield ratio of 6.6, while in the Control and the NPSi treatments it reflected N-limitation in the phytoplankton cells.

The initial C:N ratio (mean $\pm$ SD) in the copepods on Day 6 was 8.9 ± 2.0 in Exp 1 and 5.5 ± 0.3 in Exp 2. In Exp 1 there were no significant differences between treatments and no trends with time were detected (rm ANOVA, Fig. 8c). In Exp 2 the C:N ratio in the copepods increased with time in both treatments (Fig. 8d), but it increased more in the NPSi treatment, reflecting the N-limitation of the phytoplankton in this treatment.

The phytoplankton N:P ratio (only available for Exp 2), did not differ significantly between treatments throughout the experiment (rm ANOVA), although it was usually lower in the NPSi treatment reflecting N-limitation (Fig. 9a). During the experiment it steadily decreased with time in both treatments from ca. 14.5 to ca. 12.5. This shows that even in the NP treatment N became increasingly limiting for algal growth relative to P in the course of the experiment. The copepod N:P ratio did not show significant differences between treatments or any trends with time (rm ANOVA, Fig. 9b).

Discussion

C:N:P ratios

Our study shows that N-limitation in phytoplankton cells can be transferred to their copepod grazers even if grazers often possess physiological solutions to the problem of excess C in their diet by not assimilating C across the gut wall or by disposing assimilated C through respiration or extracellular release of organic C-compounds (Sterner & Hessen, 1994). In the N-limited NPSi treatment of Exp 2, copepod C:N ratios became higher than in the Si-limited NP treatment, and this corresponded to the phytoplankton C:N ratios in the respective treatments. Our copepod C:N ratios (5–11) were in the range of previously reported values for marine and brackish-water copepods, and our N:P ratios (16–22) were close to previously reported values for copepodites (Gismervik, 1997; Walve & Larsson,

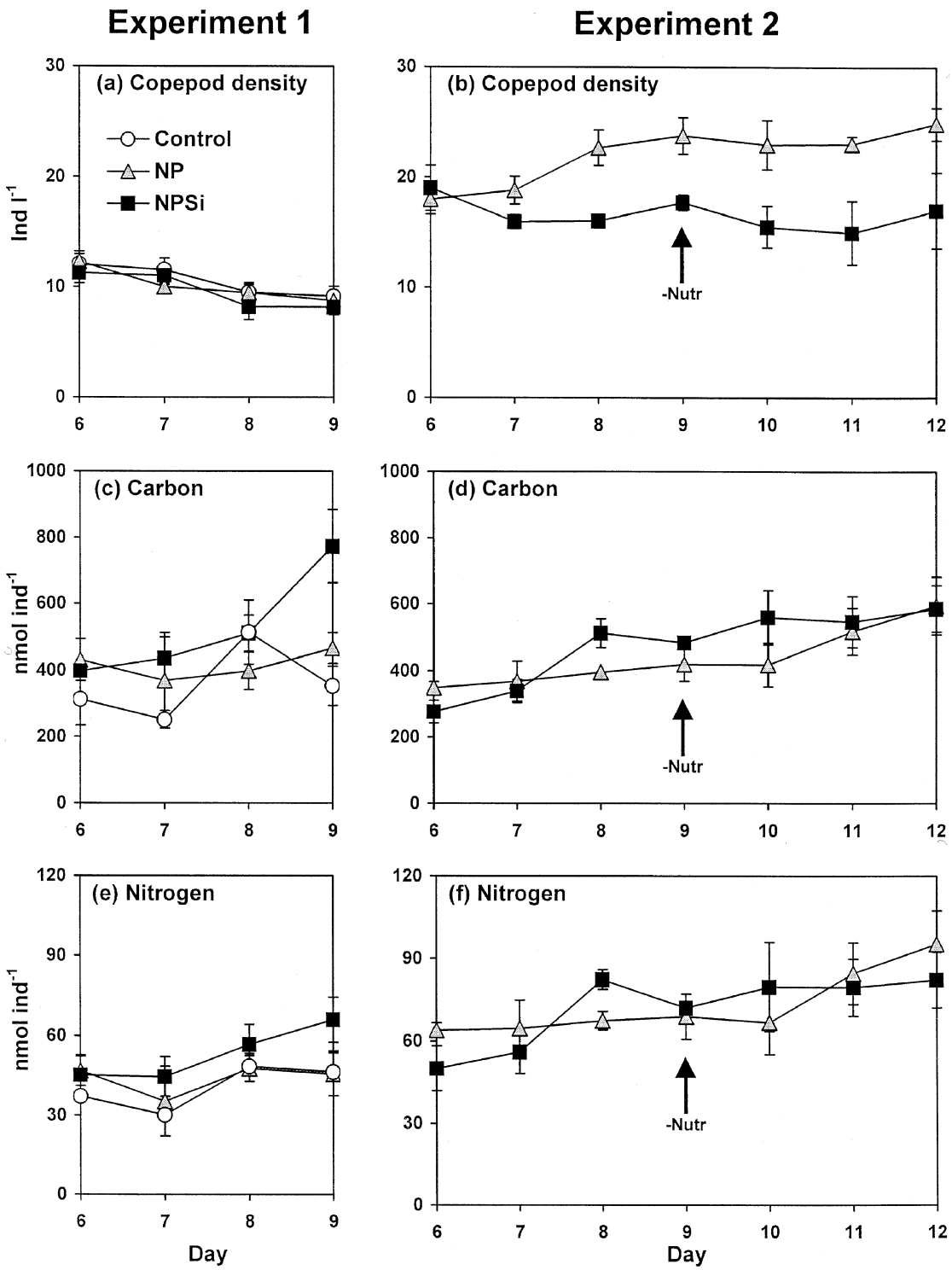

Figure 7. Copepod density and nutrient content ($n = 3$) during the two mesocosm experiments: (a, b) number of copepod individuals per litre, (c, d) carbon content, (e, f) nitrogen content. Arrows indicate the day of the termination of nutrient additions (−Nutr). Error bars represent 1 standard error of the mean.

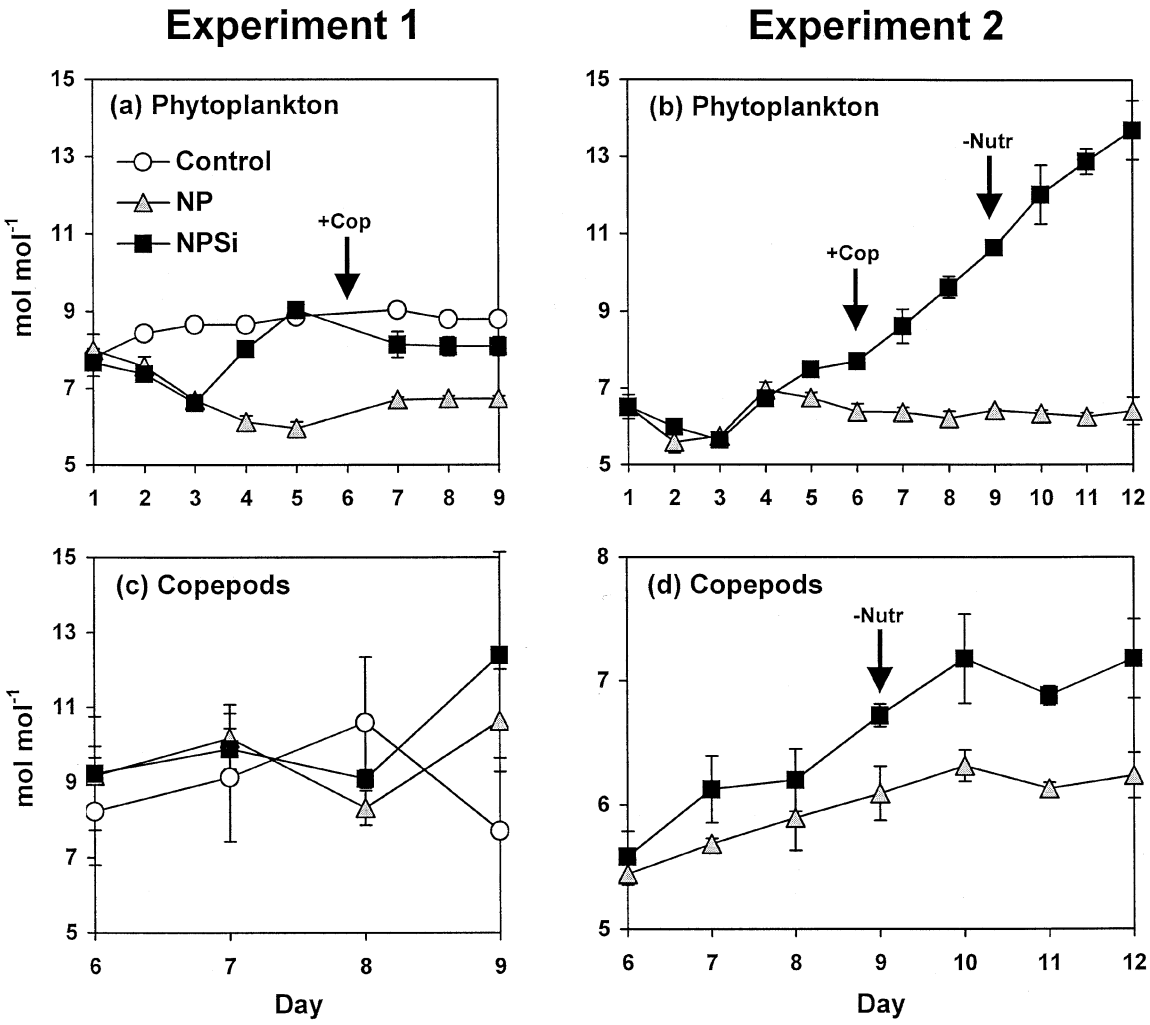

Figure 8. C:N ratios ($n = 3$) during the two mesocosm experiments in phytoplankton (a, b) and copepods (c, d). Arrows indicate the days of copepod additions (+Cop) and the termination of nutrient additions (−Nutr). Error bars represent 1 standard error of the mean.

1999; Carrillo et al., 2001; Pertola et al., 2002). Generally, high C:N ratios in copepods are considered indicative of storage of C-rich compounds like starch or lipids, later to be used for metabolism or reproduction (Sterner & Hessen, 1994; Evanson et al., 2000; Hays et al., 2001). Koski (1999) reported that C:N ratios of the copepod *Eurytemora affinis* increased with increasing food supply. This may also have been the case in our Exp 2 because in both types of fertilised mesocosms the copepods had a high food supply while simultaneously copepod size (C ind^{-1}) and C:N ratios increased in the course of the experiment. However, the latter was not observed in our Exp 1 despite a high food supply in the NP and NPSi treatments.

Copepod growth

The observed difference in copepod density development between the N- and Si-limited mesocosms in Exp 2 may have been an effect of N-limitation. If this was a direct effect of N-limitation (malnutrition) or an indirect effect triggered by other compounds in the diet or other interactions between phytoplankton and copepods cannot be extracted from the present study. The copepods that were successful in increasing their numbers by ca. 40% (Exp 2, NP treatment) had lower C:N ratios than the ones with stable density (Exp 2, NPSi treatment). In the growing copepod assemblage the C:N ratio was 5.5–6 in copepods grazing on Redfield ratio phytoplankton (Exp 2, NP treatment). In the stable copepod assemblage the C:N ratio increased to

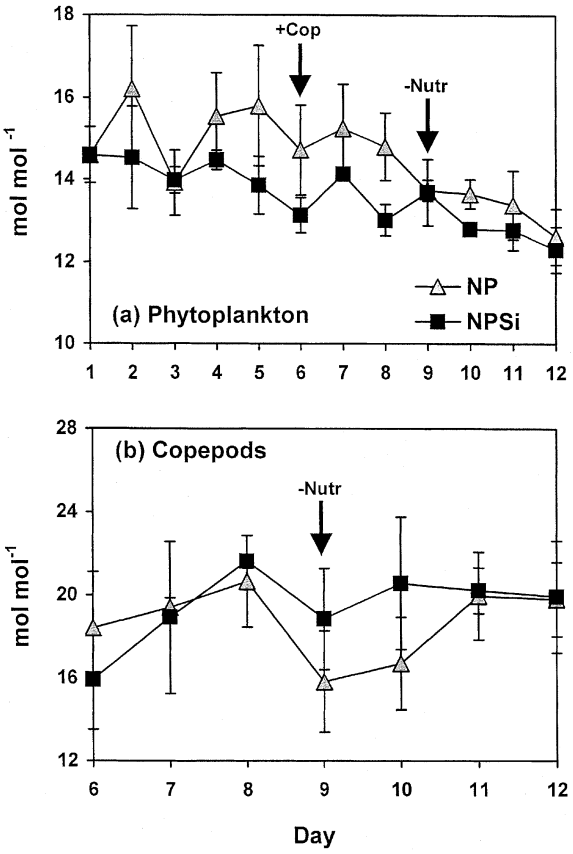

Figure 9. N:P ratios ($n = 3$) during Experiment 2 in phytoplankton (a) and copepods (b). Arrows indicate the days of copepod additions (+Cop) and the termination of nutrient additions (−Nutr). Error bars represent 1 standard error of the mean.

ca. 7 in the course of the experiment (Exp 2, NPSi treatment), and in the declining copepod assemblage it was around 9 throughout the experiment (Exp 1, all treatments). The increase in copepod density can only be explained by growth of eggs/nauplii that were present in the sieved seawater at the start of the experiment (Day 1). If high copepod C:N ratios were an expression of high lipid reserves, this did not stimulate the growth from eggs/nauplii to copepodites in our experiments. An alternative explanation for the differences in copepod density development between the two experiments could be low initial abundances of eggs/nauplii in Exp 1.

Diatoms and copepod grazing

The taxonomic composition of the phytoplankton in our experiments may have transferred an indirect negative effect of N-depletion to copepod development. The total dominance of heavily silicified diatoms in the N-limited mesocosms contrasted with flagellate dominance in the Si-limited mesocosms. Diatoms are a diverse phytoplankton group and they are highly competitive in most aquatic environments as long as dissolved silicate is available (Snoeijs, 1998), since they have an obligate need for Si in the form of dissolved $SiO_2 \cdot nH_2O$ for the formation of their frustules. Therefore, silicate is of large importance as a structuring agent in phytoplankton communities. The dominance of non-silicified phytoplankton taxa in Si-depleted systems, as we found in the present study, has previously been described by, e.g. Egge & Aksnes (1992) and Duarte et al. (2000). When the diatom community in the NPSi treatment of our Exp 2 was rapidly growing after Day 5, the Si-uptake was more than three times the N-uptake (dissolved Si decreased while DIN increased), which supports the observations of Kristiansen & Hoell (2002) that Si uptake is usually undersaturated compared to the Si:N ratio of 1:1 which is often mentioned for diatom growth (e.g. Redfield et al., 1963).

Transfer ratios based on pigment analyses of the phytoplankton and copepods from the mesocosm experiments reported here indicate selective grazing (Van Nieuwerburgh et al., unpublished). Diatoms were consumed more in the NP treatment than in the NPSi treatments. We also found that grazing on Si-limited diatoms (as major component of a varied diet in our NP treatment: Van Nieuwerburgh et al., unpublished) appears to be beneficial for copepod development. One possible explanation is that the diatoms in the NPSi treatment were too large to ingest and more heavily silicified than the diatoms in the NP treatment. Diatom cells with thick silica frustules are probably less palatable than cells with thin ones. Another possibility is an increased growth of heterotrophic cells in the phytoplankton fraction of the Si-limited mesocosms (NP treatments), which may have had a positive effect on copepod development as some copepod species seem to prefer heterotrophic prey (Nejstgaard et al., 2001). Several studies have reported grazing selectivity against diatoms (Strom & Welschmeyer, 1991; Kleppel et al., 1991). It has been proposed that some diatoms are of inferior nutritional quality because they lack some essential lipids (Kleppel, 1993; Jónasdóttir & Kiørboe, 1996), while others have claimed the nutritional superiority of diatoms (Brown et al., 1997). Further explanations for avoidance of grazing on diatoms include the excretion of toxins by dense diatom communities, which have been shown to

have direct detrimental effects on copepod reproduction (Miralto et al., 1999; Tang & Dam, 2001). The slower development of eggs/nauplii to copepodites in our diatom-dominated N-limited mesocosm in Exp 2 may have been caused by diatom toxins. In previous mesocosm experiments at Hopavågen (the same site as our Exp 2), Gismervik et al. (2002) found that diatom growth and nauplii mortality were enhanced by half the daily N dose we used in our experiments at Si:N:P = 16:16:1. Also Nejstgaard et al. (2001) recorded negative effects on copepod growth in a diatom bloom (Si:N:P = 16:16:1) in other mesocosm experiments on the Norwegian coast (Bergen). Similar to these studies, our results with Si:N:P = 48:16:1 show that a heavy diatom bloom can hamper copepod growth. Thus, copepod populations can be strongly regulated by seawater Si:N ratios via diatoms. High Si:N ratios provide diatoms with possibilities to escape from grazing (large species, heavy silicification, excretion of secondary metabolites), leading to the accumulation of algal biomass without transfer to higher trophic levels.

Consequences for food web dynamics

Our results would imply that Si-limitation is a better condition than N-limitation for copepod development and thereby for ecosystem productivity. However, long-term Si-limitation in nature will lead to fundamental changes in the food-web, e.g. with harmful algal blooms (Lassus et al., 1995). Our results show that increases in DIN with eutrophication may increase copepod populations, but when dissolved silicate levels are very high as well, copepods may be growth-inhibited. The latter is similar to results obtained in previous laboratory experiments in which growing, nutrient-saturated diatoms were shown to have negative effects on copepod growth (Ban et al., 1997). However, in nature diatom blooms tend to occur at dissolved Si:DIN ratios of 1:1 and copepod recruitment usually occurs in a post-bloom phase (Turner et al., 1998; Miralto et al., 1999). As several large data sets of field measurements show that diatom blooms and copepod abundances are strongly correlated (Turner et al., 1998; Irigoien et al., 2002), detrimental effects of diatom blooms on copepods in nature are probably only temporary and part of the natural dynamic interactions between diatoms and copepods.

Acknowledgements

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Effects of grazing and excretion by pelagic mysids (*Mysis* spp.) on the size structure and biomass of the phytoplankton community

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Abstract

The aim of this study was to determine the effects of pelagic mysids (*Mysis mixta* and *M. relicta*) on the biomass and size-structure of the phytoplankton community during the period following the spring bloom. Mysids excreted phosphate (4.5 ± 0.7 nmol ind^{-1} h^{-1}) and ammonium (123.6 ± 31.6 and 45.0 ± 3.2 nmol ind^{-1} h^{-1}) and increased the total chlorophyll-*a* concentration of phytoplankton slightly. However, the presence of mysids affected different size-classes of phytoplankton differently. Mysids mainly grazed on large-sized (>10 μm) phytoplankton cells. Small-sized (<10 μm) algal cells avoided grazing, gained a competitive advantage and were able to utilize the nutrients excreted by mysids. According to this study, both top-down and bottom-up mechanisms simultaneously mould the structure of the phytoplankton community. A large zooplankton biomass might promote the increase of small flagellates by a combination of repleting nutrient stores, selective grazing on large algal cells and heavy predation on protozoa which, consequently, might have a cascading effect on the most favoured protozoan food source, small flagellates.

Introduction

Mysis mixta (Lilljeborg) and *M. relicta* (Lovén) are pelagic mysid shrimps in the Gulf of Finland. They start breeding in late autumn and release the juveniles after ice break-up, when the spring bloom of phytoplankton is most intense (Rudstam et al., 1986; Salemaa et al., 1986; Väinölä, 1986; Rudstam & Hansson, 1990).

The diet of mysids varies seasonally since the availability of different planktonic food item groups varies considerably during the growth season. The diet changes also as the juveniles mature, since small-sized mysids cannot capture actively moving zooplankton as well as larger adult individuals can (Cooper & Goldman, 1980; Viherluoto et al., 2000). In spring, mysids feed almost solely on phytoplankton (ca. 70-80%; Viherluoto et al., 2000). Spring phytoplankton is a highly suitable nutrition source for the juveniles, since the cell size is small (<100 μm) and during the bloom, plenty of it is available. Of the main phytoplankton groups, mysids feed most on diatoms (Diatomophyceae), which in June constitute 58% of the total phytoplankton consumed (Viherluoto et al., 2000). They also feed on dinoflagellates (Dinophyceae), green algae (Chlorophyceae) and blue-green algae (Cyanobacteria; Viherluoto et al., 2000). The use of zooplankton increases as summer proceeds, when mysids grow larger and are thus more able to utilize the increased biomass of zooplankters (Adare & Lasenby, 1994; Viherluoto et al., 2000).

Nutrients, which have accumulated in the water during winter, are depleted from the epilimnion during the phytoplankton spring bloom in the Baltic Sea, and the concentrations of inorganic nutrients become very low (Tamminen, 1990). During summer, the production is mainly based on the regeneration of nutrients

(Tamminen, 1990; Lignell et al., 1992). The availability of nutrients is linked to the mineralization rate, since the nutrient concentrations in the water would support primary production only for an hour or two (Tamminen, 1990). Some new nutrients do arrive in the ecosystem also during summertime, through e.g. physical mixing processes and nitrogen fixation. In autumn, as the water column mixes, nutrients from the hypolimnion arrive, and the importance of regeneration decreases (Tamminen, 1990). With regard to nitrogen, the new production is based on the use of nitrate (NO_3^-) and molecular nitrogen (N_2); the regenerated production is based on ammonium (NH_4^+) and dissolved organic nitrogen (DON; Dugdale & Goering, 1967). The zooplankton plays an important role in nutrient regeneration, since it releases both inorganic and organic nutrients while handling and feeding (sloppy feeding), by excretion and as a result of the decay of faecal pellets (Lampert, 1978; Raymont, 1983; Banse, 1995).

The aim of this paper is to study the effects of pelagic mysids on the biomass and size structure of the phytoplankton community during the period following the spring bloom, when phytoplankton is still abundant in the water column, as the bloom sinks towards the bottom. We wanted to study whether mysids feed equally on different phytoplankton size classes and if the nutrients excreted by mysids affect the growth of phytoplankton in the northern Baltic Sea. The hypothesis was that the mysids would graze more effectively on large-sized (diameter >10 μm) cells or colonies, such as diatoms. Thus, small-sized phytoplankton could avoid grazing and gain a competitive advantage for nutrients excreted by the mysids. This process could lead to an increase of small-sized algae up to the point of a mass occurrence or bloom forming. We wanted to show the general impact of mysids on the planktonic community through both top-down (grazing) and bottom-up regulation (nutrient recycling).

Materials and methods

Excretion experiments

The excretion experiments were conducted in May, June and August 2000. The mysids (*M. mixta* and *M. relicta*) were collected with an epibenthic sled from Längden (59°45′ 40″ N; 23°13′ 90″ E), an open deep-sea area near the Hanko Peninsula at the entrance to the Gulf of Finland, or with a large zooplankton net from the 125 m deep monitoring station SR5 (61°13′ N; 19°97′ E) in the Bothnian Bay. They were kept in filtered seawater and fed with commercial fish food (Aqua Win, Kerox Oy; Längden) or with natural zooplankton (SR5). The length of the mysids was 2 to 3 cm. Seawater was filtered through a GF/F glassmicrofiber filter (Whatman) into experimental containers of 250 ml (Längden) or 60 ml (SR5), and one mysid, after measuring its length, was added to each container. Containers without mysids served as controls. All the containers were incubated at 10 °C (Längden) or 3 °C (SR5) for ca. 12 h, after which the concentrations of dissolved nutrients (NH_4^+ and PO_4^{3-}) were measured (Grasshoff, 1976; QuikChem Method, 1998). The amount of excreted nutrients in the containers with the mysids was calculated by subtracting the average concentration of the respective nutrient in the control containers from the nutrient concentration in the experimental container. Altogether 64 individual animals were tested. The data was tested for statistical significance using a *t*-test, after $\log(x + 1)$ transformation of the data.

Community experiments

The water for the experiments was collected from Storgadden (59°47′ N; 23°19′ E; depth 46 m) or Storfjärden (59°57′ N; 23°16′ E; depth 33 m), which are situated southeast of the Hanko Peninsula. The natural phytoplankton community (filtered through a 100-μm net) was collected with a Limnos sampler from ca. 1 m depth and the water temperature was recorded. The temperature of the climate room was set at the beginning of each experiment according to the water temperature at the time of collection (6 to 8 °C). The experimental containers were illuminated from above by fluorescent lamps with 60 μE m^{-2} s^{-1}, corresponding to the light environment at about 5 m depth during the day. The light/dark period was 18/6 h.

The mysids were collected from Längden with an epibenthic sled, kept in an aquarium filled with seawater and fed with commercial fish food. The light/dark period was 18/6 h and the temperature of the room was ca. 10 °C. The mysids were starved for 24 h before starting the experiments. The size of the mysids was 1 to 3 cm.

Five experiments were conducted during May and June 2000, with three replicates in each treatment. 2 l transparent glass bottles were used as experimental containers. The treatments were mysid addition (five

mysids), nutrient addition (NH_4Cl-N 2.9 nmol l^{-1} and Na_2HPO_4-P 0.32 nmol l^{-1}) and control (without nutrient additions). The containers were incubated in the climate room for three days. To facilitate gaseous exchange, the mysid containers were closed only with cotton plugs. On each day of the experiment, two 100 ml samples for chlorophyll-*a* analysis were taken from each container. One sample was used for total chlorophyll-*a* and the other sample was filtered through a 10-μm polycarbonate filter (Poretics) to remove particles larger than 10 μm. From each chlorophyll-*a* sample, two 50 ml samples were filtered onto GF/F filters, which were then placed into 10 ml 96% ethanol. The samples were ultrasonicated for 10 min and chlorophyll was extracted for at least 6 h. Before measuring the samples were filtered through GF/F filters to remove any impurities. Chlorophyll-*a* concentrations were measured with a Shimadzu spectrofluorometer calibrated with pure chlorophyll-*a* (Sigma Chem. Co., U.S.A.), using 96% ethanol as the 0-sample.

If the data fulfilled the assumptions of parametric tests (in some cases after $\log(x+1)$ transformations of the data), one-way analysis of variance (ANOVA) and *t*-test were performed. Otherwise Kruskal–Wallis one-way nonparametric analysis of variance and Mann-Whitney *U*-test were performed. The results presented in this paper are combined from all five experiments.

Results

The excretion of phosphorus by mysids was not significantly different at the two locations (Längden and SR5; *t*-test: $t = 1.568$, $df = 47$, $p > 0.05$). Thus, the results presented here are combined for the two locations. However, the excretion of ammonium varied significantly (*t*-test: $t = 3.955$, $df = 48$, $p < 0.001$) between the two locations. The mysids excreted (mean ± SE) phosphate (PO_4^{3-}) 4.5 ± 0.7 nmol ind^{-1} h^{-1} and ammonium (NH_4^+) 123.6 ± 31.6 nmol ind^{-1} h^{-1} (Längden) and 45.0 ± 3.2 nmol ind^{-1} h^{-1} (SR5). The mean N/P ratios of the excretion were 2.36 (Längden) and 9.49 (SR5).

The combined addition of the two main nutrients (N and P) had an immediate effect on total chlorophyll-*a* value (Fig. 1a). This effect levelled after the second day. The increased chlorophyll-*a* was mostly due to the increase in larger phytoplankton fraction, but also small-sized algal fraction showed a positive response (Figs 1b, c). Mysid addition increased the total chlorophyll-*a* concentration of the phytoplankton compared to control (day 3, *t*-test: $t = 3.635$, df = 22, $p < 0.001$; Fig. 1a), but its effect on different size classes of phytoplankton varied greatly. Mysids obviously grazed on large-sized (>10 μm) phytoplankton cells, as chlorophyll-*a* concentration of that size-fraction decreased significantly (day 3, *t*-test: $t = 3.98$, df = 23, $p < 0.001$; Fig. 1b). At the same time, the presence of mysids increased the

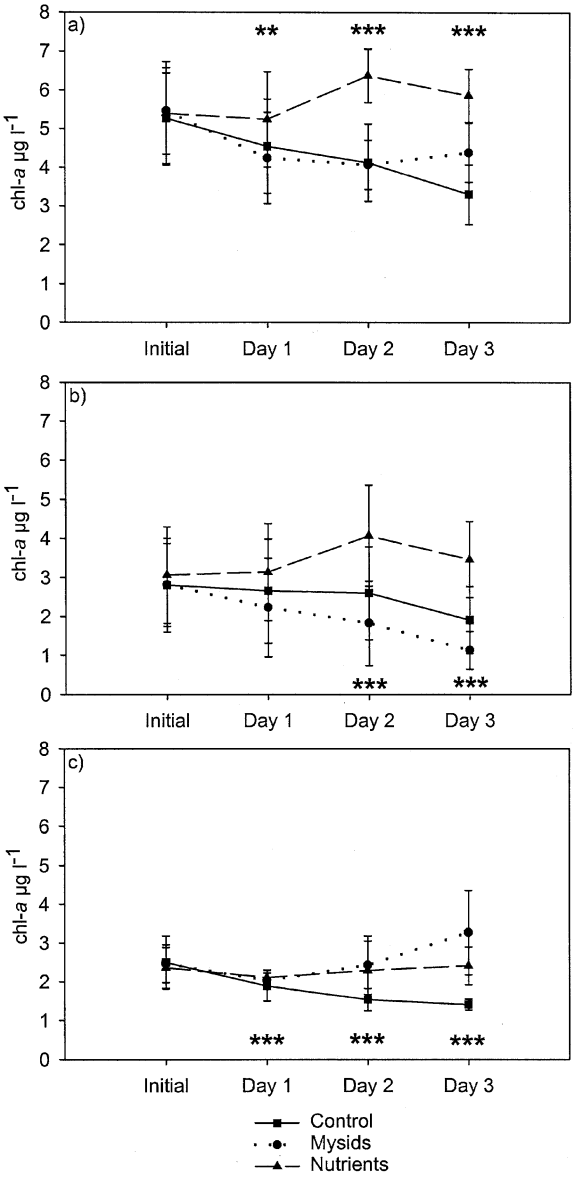

Figure 1. Chlorophyll-*a* concentration (mean ± SD) in three treatments. (a) Total, (b) in >10 μm size fraction, (c) in <10 μm size fraction. ** = $p < 0.01$, *** = $p < 0.001$, denotes the results of ANOVA or Kruskal–Wallis.

chlorophyll-*a* concentration of small phytoplankton (<10 μm) significantly, even compared to the nutrient addition treatments (day 3, Mann–Whitney *U*-test: $p < 0.05$; Fig. 1c).

Discussion

Mysids actively excreted inorganic nutrients, ammonium and phosphate in the ratio of 2.36–9.49, which is in the same range as *M. relicta* in Lake Michigan (Madeira et al., 1982), where the measured N/P-ratio was 5.7, ammonia excreted at the rate of 0.17 μg N mg dw^{-1} h^{-1} and phosphate 0.03 μg N mg dw^{-1} h^{-1}, which would supply 1–10% of the nitrogen and phosphorus required by thermocline-associated phytoplankton. Our N/P ratios also correspond with the N/P ratios in the two most common copepods in the northern Baltic Sea, *Acartia bifilosa* (4.0) and *Eurytemora affinis* (4.57; M. Koski & M. Viitasalo, unpubl.), which formed a major part of the diet of the mysids in the excretion experiments conducted at SR5. Both nitrogen and phosphorus together are more efficient in enhancing primary production than either of the nutrients alone during early summer (Kivi et al., 1993). Thus the excretion of mysids was likely to have a bottom-up effect on the growth of phytoplankton. However, our excretion values are near maximum, due to the nutritional history of the animals, i.e. they were fed with food which was high in protein (zooplankton or commercial fish food). This, together with the lower temperature at the site, may also explain the lower ammonium excretion rates of mysids collected from SR5. Temperature, however, has been found not to have an effect on the amount of nitrogen excreted by zooplankton, but it depends more on the quantity and quality of the available food, or other environmental or physiological factors (Jacobsen & Comita, 1976; Seale & Boraas, 1982). When plenty of food is available, the zooplankton utilizes mostly carbohydrates for the production of energy and saves proteins for growth, and very little ammonium is excreted. When food is scarce, proteins are catabolized to energy, which leads to increased excretion of ammonium and decreased growth rate (Martin, 1968; Mayzaud, 1976). However, Weisse & Rudstam (1989) have shown that temperature does affect the excretion rates of ammonia and phosphate in *Neomysis integer*, though not the N/P ratio of the excreta. Their experiments gave N/P ratios of 7–26, ammonia excretion of 2–7 μmol g dw^{-1} h^{-1} and phosphate excretion of 0.2–1 μmol g dw^{-1} h^{-1}, but these values are not directly comparable to ours since the mysids in their experiments were starved several hours prior to the experiments.

Pelagic mysids migrate diurnally between the benthos and the water column (Aneer, 1980), so excretion occurs not only in the euphotic zone. Thus, not all nutrients excreted are available to the phytoplankton. Mysids also transfer phytoplankton biomass away from the euphotic zone, when they migrate back to the bottom in the morning with their gut full after feeding (Madeira et al., 1982). Mysids are found in the euphotic zone during night time, when no light assimilation occurs, but the phytoplankton is able to take up nutrients also during darkness (Harvey, 1963). The patchy distribution of mysids (Haury et al., 1978) results in an uneven distribution of the excreted nutrients as well. Thus it is still premature to evaluate the real effect of mysid remineralization to bottom-up dynamics, but our results indicate a potential mechanism of nutrient replenishment during the summer minimum.

Excretion experiments are technically difficult to conduct. The quality of the experimental water changes constantly; the oxygen concentration decreases, carbon dioxide and ammonium accumulate and the ionic concentrations and pH also change. These changes and the handling of mysids could cause stress and thus a change in excretion rates (Ikeda, 1977). High external ammonia concentration probably decreases the diffusion of ammonia from the tissues and can also stimulate excretion of urea (Mullin et al., 1975; Seale & Boraas, 1982). In addition, the organic or inorganic nutrients dissolved from the faecal pellets during the experiment may have affected the results. We did monitor the oxygen level during the experiments and found declining, but not critical, oxygen values. Taking into account the drawbacks of the experimental system, we give our results as preliminary and encourage further studies on the subject, whilst taking into account size variability and the nutritional history of mysid populations.

According to our study, Baltic pelagic mysids do have a different effect on phytoplankton biomass in different size classes. The different succession of chlorophyll-*a* concentrations in different size fractions supports the hypothesis of size-selective feeding (Viherluoto et al., 2000). In containers with mysids, the chlorophyll-*a* concentration of large (>10 μm) phytoplankton decreased significantly compared to other treatments. Thus it can be concluded that mysids fed on this size class. In addition, Bowers & Gross-

nickle (1978) observed in their experiments in Lake Michigan that adult or juvenile *Mysis relicta* did not significantly graze on algal cells smaller than 10 μm and they grazed most effectively on the size class of over 53 μm. In our experiments, the larger phytoplankton fraction consisted mainly of diatoms (*Skeletonema costatum* and *Chaetoceros* spp.), small dinoflagellates and small colonial blue-green algae, all of which are considered to be suitable food sources for mysids, and which have previously been shown to be heavily grazed upon by mysids (Viherluoto et al., 2000). Large trichal and potentially toxic blue-green algae were not present in the early summer experiments.

As small algal cells avoided grazing, they gained a competitive advantage over larger ones and were able to utilize the nutrients excreted by mysids. In particular, the high surface-to-volume ratio of small cells aids in absorbing nutrients even from low concentrations. Another beneficial factor for the success of small-sized phytoplankton is their release from the grazing pressure of pelagial ciliates by mysid predation on ciliates. This is a common cascading effect in Baltic summer communities, in which ciliates are tightly controlled by crustacean predators (Kivi et al., 1993). The smallest size-fraction consisted mainly of small flagellated cells from several systematic groups (Chrysophyceae, Prasinophyceae, Cryptophyceae and Prymnesiophyceae). However, their similar functional characteristics, small cell size, naked cell surface and active swimming, make all of them optimal food source for protozoan grazers. Also, Kivi et al. (1996) found in their study on Baltic mesozooplankton, that the 1 to 10 μm size-class phytoplankton increased in containers where both nutrients (nitrogen and phosphorus) and crustacean zooplankton were added. This size-class avoided mesozooplankton grazing due to their small size and gained from both the nutrient addition and the nutrient regeneration by the animals.

Conclusions

According to our results, mysids regenerate nutrients along with other zooplankton and thus maintain phytoplankton production. At the same time, the size selectivity of the feeding of the mysids moulds the size structure of the phytoplankton community in the Baltic Sea, favouring small-sized phytoplankton, mainly small flagellates, and reducing larger phytoplankton. Thus both top-down and bottom-up mechanisms work simultaneously between these trophic levels.

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Food and habitat choice of the isopod *Idotea baltica* in the northeastern Baltic Sea

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Key words: Baltic Sea, habitat choice, food choice, experiment, *Fucus vesiculosus*, *Idotea baltica*, *Pilayella littoralis*

Abstract

The isopod *Idotea baltica* is the most important benthic herbivore in the Baltic Sea. There exists a significant correlation between the distribution of the adult isopod and the belts of bladder wrack *Fucus vesiculosus*. However, following the eutrophication induced blooms of the filamentous macroalga *Pilayella littoralis* and the disappearance of *F. vesiculosus* a notable increase in idoteid abundances has been observed. The aim of this paper was (1) to evaluate experimentally whether *F. vesiculosus* provides either food, shelter or both to the isopods and (2) to estimate the role of associated filamentous algae in the habitat selection process. Amongst six abundant macroalgal species, about 50% of isopod population was attracted to *F. vesiculosus* covered with the filamentous algae *P. littoralis*. The majority of the remaining part of the population was either swimming freely or attracted to non-epiphytic *P. littoralis*. When both live algae and artificial substrata were provided, *P. littoralis* growing on artificial substrata was clearly preferred by the isopods over epiphyte-free *F. vesiculosus*. In the grazing experiment where *I. baltica* was allowed to choose between *F. vesiculosus* and *P. littoralis* the latter contributed practically 100% of the diet of the isopod. The results indicate the importance of *P. littoralis* as a food item and *F. vesiculosus* as a shelter for *I. baltica*.

Introduction

The euryhaline *Idotea baltica* (Pallas) is originally a littoral and sublittoral crustacean of tidal shores with a characteristic pattern of distribution correlated with tidal level, exposure and salinity variations of the habitat. It has a wide, almost cosmopolitan distribution, and its geographical races have been described (Naylor, 1955; Sywula, 1964). The species has also penetrated into the brackish waters of the Baltic Sea (Salemaa, 1978, 1979) where it is ranked among the most important necto-benthic herbivores (Schaffelke et al., 1995).

Habitat choice of animals is determined by several factors such as food, shelter and access to mates. Vegetation is crucial for herbivores both as a food source and as protection from predation (Puttman, 1986). In the Baltic Sea, *I. baltica* is common in the *Fucus vesiculosus* L. and *Cladophora glomerata* (L.) Kütz. belts (Jansson, 1974; Haahtela, 1984), but it also occurs on *Zostera marina* L. dominated soft bottoms (Salemaa, 1978; Boström & Bonsdorff, 1997). *I. baltica* is omnivorous, eating benthic microalgae, filamentous algae, macroalgae, detritus, small invertebrates and even its conspecifics (Naylor, 1955; Ravanko, 1969; Sywula, 1964; Nicotri, 1980; Robertson & Mann, 1980; Franke & Janke, 1998). Despite its omnivory *I. baltica* is quite selective within food categories (Salemaa, 1978; Schaffelke et al., 1995; Jormalainen et al., 2001). Its dietary choice involves the selection between different species (Schaffelke et al., 1995; Schramm et al., 1996), but likely between different parts of algal thalli (Salemaa, 1987). However, in the northern Baltic Sea, *F. vesiculosus* is considered as the main source of food for *I. baltica* (Salemaa, 1987; Jormalainen et al., 2001).

Following coastal eutrophication, mass developments of filamentous algal species have been observed

in the Baltic Sea during the last decades (Bonsdorff et al., 1997; Bäck et al., 2000; Norkko et al., 2000; Vahteri et al., 2000; Lehvo & Bäck, 2001). There exists circumstantial evidence that after the extensive growth of *P. littoralis* and the disappearance of *F. vesiculosus* the density of idoteids has increased hundreds of times (Kotta et al., 2000). This suggests that other algae than *F. vesiculosus* would be the primary diet for these isopods. The literature does not provide a definite answer if *I. baltica* will select *F. vesiculosus* for shelter or for food, because experiments considering the microhabitat choice of idoteids usually involved only a single macroalgal species (e.g. Salemaa, 1987; Merilaita & Jormalainen, 2000). Therefore, the aim of the present study was to show the importance of substrate structure and food quality of different macroalgal species on the habitat selection of *I. baltica*. We hypothesized that when the density of filamentous algae is low, i.e. in low eutrophicated conditions, *F. vesiculosus* provides both food and shelter for *I. baltica*. However, under eutrophicated conditions, filamentous algae are expected to be the prime food for *I. baltica*, and the occurrence of filamentous algae will significantly modify the isopods habitat choice. In eutrophicated conditions, filamentous algae may indirectly offer shelter for idoteids as their predators seem to avoid areas of with mass blooms of annual algae (Salemaa 1979, 1987).

Material and methods

Sampling of *I. baltica* was performed in Kõiguste Bay, northeastern Baltic Sea in June 2000. Adult *I. baltica* (mean ± SE: 17 ± 1 mm) were collected from a shallow hard bottom area, at 1–3 m of depth, within *F. vesiculosus* fronds by shaking the algae.

Six different macroalgal species were used in the experiments: the brown algae *F. vesiculosus*, *Chorda filum* (L.) Stackh. and *Pilayella littoralis* (L.) Kjellm., the red alga *Ceramium tenuicorne* (Kütz.) Waern and the green algae *C. glomerata* and *Enteromorpha intestinalis* (L.) Nees. *F. vesiculosus* has a bush-like structure, *C. filum* consists of coarse filaments and the other four species are fine filamentous algae. *E. intestinalis* and *C. glomerata* are known to cause extensive macroalgal blooms in shallow water and *P. littoralis* in the sublittoral (Bonsdorff et al., 1997; Bäck et al., 2000; Norkko et al., 2000; Vahteri et al., 2000; Lehvo & Bäck, 2001). Macroalgae were collected at the same site as the isopods. The lower and upper parts of *F. vesiculosus* thallus differ in terms of their nutritional and protective qualities (Tuomi et al., 1989; Carlson, 1991). Therefore, we used only those specimens of *F. vesiculosus*, which had about equal shares of younger apical and older basal thallus parts.

Four types of artificial substrata made of polypropylene, simulating plants and algae, were used: *F. vesiculosus*, *Furcellaria lumbricalis* (Huds.) J.V. Lamour., *Z. marina* and filamentous alga. The artificial *F. lumbricalis* was more fragile and its texture was finer compared with artificial *F. vesiculosus*. *Z. marina* had narrow non-branching blades and filamentous alga represented the type of *C. glomerata* and *P. littoralis*. These artificial substrata represented the prevalent morphological forms of plants and algae that *I. baltica* may encounter in the study area.

The habitat choice of *I. baltica* was studied in five different experiments. In the first experiment we used *F. vesiculosus*, *C. filum*, *C. glomerata*, *C. tenuicorne*, *E. intestinalis* and *P. littoralis*. Prior to the experiment, the epiphytes growing on *F. vesiculosus* were removed and some fronds of *F. vesiculosus* were artificially covered with the epiphyte *P. littoralis*. In the second experiment the attractiveness of live and artificial *F. vesiculosus* was compared to artificial *F. vesiculosus* covered with epiphytes. If *I. baltica* would select live *F. vesiculosus*, this would indicate its importance in the diet of the isopod. On the other hand, if there was no significant difference in habitat choice of *I. baltica* between live and artificial *F. vesiculosus*, then the alga would serve as a shelter for *I. baltica*. This experiment also tests if epiphytic *P. littoralis* and *C. glomerata* will increase the attractiveness of artificial *F. vesiculosus* as a substrate. The third experiment was designed to study the habitat selection of *I. baltica* in less eutrophicated conditions. To simulate this, epiphytic *P. littoralis* was removed from *F. vesiculosus*. Live and artificial *F. vesiculosus* and artificial *F. vesiculosus* covered with the epiphyte *C. glomerata* were provided to *I. baltica*. In the fourth experiment, epiphyte-free live *F. vesiculosus*, artificial *F. lumbricalis* and artificial *F. lumbricalis* covered with epiphytic *C. glomerata* and *P. littoralis* were provided to *I. baltica*. *F. lumbricalis* is known to be an alternative substrate for *I. baltica* in the case of scarcity of *F. vesiculosus* (Kotta et al., 2000). This experiment tests the attractiveness of live *F. vesiculosus* in relation to another algal morphology. This experiment also shows whether the occurrence of epiphytes will affect the habitat selection of *I. baltica*. The fifth experiment included four types of artificial algae and live *F. vesi-*

culosus. The aim of the experiment was to demonstrate the role of algal morphology on the habitat selection of *I. baltica*. This experiment also tests whether live *F. vesiculosus* is favoured over artificial algae.

In each of the five experiments, the habitat choice of *I. baltica* was studied in nine replicate 100 l aquaria with a light regime similar to the field conditions. The aquaria received running seawater at a flow rate of 3 l h^{-1}. The water was taken from 2 m depth nearby Kõiguste Marine Biological Laboratory. In each aquarium macroalgae and ten *I. baltica* specimens were placed. The algae were attached to the aquarium floor by pebbles. The coverage of algae and the area without vegetation in the aquaria was 40% and 60%, respectively. The number of *I. baltica* on different macroalgae and those swimming freely were recorded every hour for 24 h. During dark periods artificial red light was used during the observations. All water was exchanged and new testorganisms were collected prior to each experiment. The survival of *I. baltica* on average (± SE) 95% ± 3%. *I. baltica* showed no significant differences in habitat preference between light and dark conditions and 24 h means were used in the statistical analyses of habitat choice.

A food preference experiment was performed with *P. littoralis* and *F. vesiculosus*, the two algal species that were preferred as habitat by *I. baltica*. Freshly collected *P. littoralis* (mean ± SE: 0.53 ± 0.09 g dry weight) and *F. vesiculosus* (0.77 ± 0.06 g dry weight) were placed into three replicate 100 l aquaria. Three control aquaria contained the same algae but no isopods. Prior to the experiment, the algae were blotted dry and weighted to obtain the wet weight. Ten *I. baltica* specimens were added to each aquarium. The experiment was run for 48 hours after which wet and dry weights of the algae was determined. For dry weight, the algae were dried at 60 °C for 48 hours. The feeding rate of the isopods was estimated in terms of algal dry weight changes. These values were corrected for the weight increment due to the photosynthetic activity of the algae in the controls.

Analysis of variance (ANOVA) was performed to investigate the habitat choice of the isopods. The data were transformed to obtain a normal distribution when necessary. Prior to the analysis, Bartlett's test was used to check the assumption of homoscedasticity (Sokal & Rohlf, 1981).

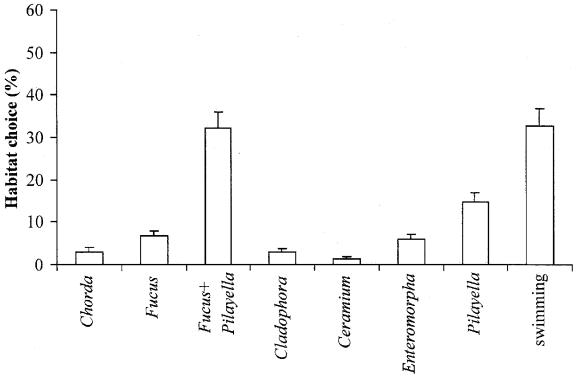

Figure 1. Habitat choice of *Idotea baltica*, expressed as the mean percentage (± SE) of the population associated with live *Chorda filum*, *Fucus vesiculosus*, *Pilayella littoralis* attached to *F. vesiculosus*, *Cladophora glomerata*, *Ceramium tenuicorne*, *Enteromorpha intestinalis*, *Pilayella littoralis* or free-swimming.

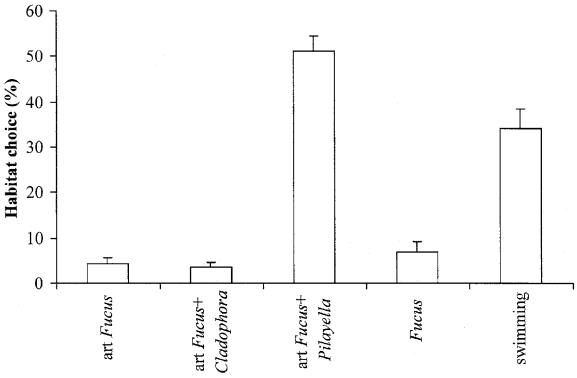

Figure 2. Habitat choice of *Idotea baltica*, expressed as the mean percentage (± SE) of the population associated with artificial *Fucus vesiculosus*, live *Cladophora glomerata* attached to artificial *F. vesiculosus*, live *Pilayella littoralis* attached to artificial *F. vesiculosus*, live *F. vesiculosus* or free-swimming.

Results

In the first habitat choice experiment including all macroalgal species, *I. baltica* preferred *F. vesiculosus* covered with *P. littoralis* as an epiphyte (32%) or non-epiphytic *P. littoralis* (15%). On average 33% of *I. baltica* were swimming freely and 20% were found on other algae of which 7% on the epiphyte-free *F. vesiculosus* (ANOVA, $p < 0.001$; Fig. 1).

In the second experiment, in which *I. baltica* were given a choice of live and artificial epiphyte-free *F. vesiculosus*, the artificial alga covered either by *C. glomerata* or *P. littoralis*, *I. baltica* significantly preferred the artificial *F. vesiculosus* with attached *P. littoralis* (51%) over the live *F. vesiculosus* (7%) (ANOVA, $p < 0.001$). There was no significant dif-

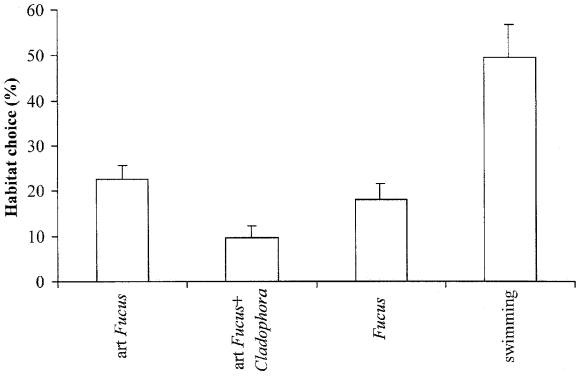

Figure 3. Habitat choice of *Idotea baltica*, expressed as the mean percentage (± SE) of the population associated with artificial *Fucus vesiculosus*, live *Cladophora glomerata* attached to artificial *F. vesiculosus*, live *F. vesiculosus* or free-swimming.

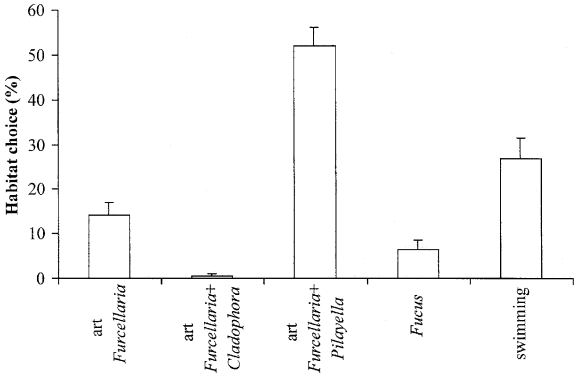

Figure 4. Habitat choice of *Idotea baltica*, expressed as the mean percentage (± SE) of the population associated with artificial *Furcellaria lumbricalis*, live *Cladophora glomerata* attached to artificial *F. lumbricalis*, live *Pilayella littoralis* attached to artificial *F. lumbricalis*, live *Fucus vesiculosus* or free-swimming.

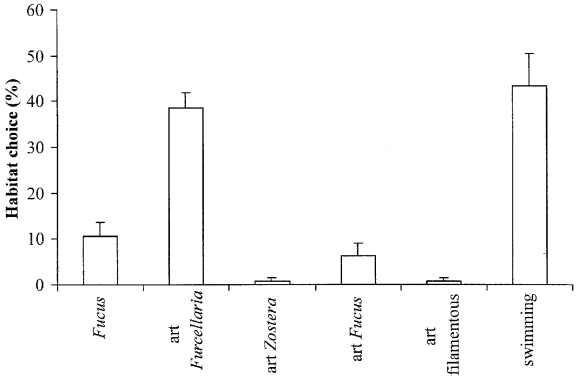

Figure 5. Habitat choice of *Idotea baltica*, expressed as the mean percentage (± SE) of the population associated with live *Fucus vesiculosus*, artificial *Furcellaria lumbricalis*, artificial *Zostera marina*, artificial filamentous alga or free-swimming.

ference in the isopod density between epiphyte-free live and artificial *F. vesiculosus* (ANOVA, $p > 0.05$; Fig. 2).

The third experiment was the same as the second, but the artificial alga covered with the epiphyte *P. littoralis* was removed. As a consequence, half of the *I. baltica* were swimming freely (50%). Similar numbers were observed on live and artificial *F. vesiculosus* (18% and 23%, respectively), and the lowest numbers on artificial *F. vesiculosus* covered with *C. glomerata* (10%) (ANOVA, $p < 0.01$; Fig. 3).

In the fourth experiment, a design similar to the second experiment was used. The artificial *F. vesiculosus* was replaced by artificial *Furcellaria lumbricalis* with a more fragile and finer texture. The habitat choice of *I. baltica* resembled the result of the second experiment, but the artificial *F. lumbricalis* was much more attractive to *I. baltica* than the live *F. vesiculosus*. Similar to the previous experiments, *I. baltica* were mostly attracted to *P. littoralis*, now attached to artificial *F. lumbricalis* (52%) (ANOVA, $p < 0.001$; Fig. 4).

In the last experiment of habitat choice, all live macroalgae were removed except for *F. vesiculosus*. The majority of *I. baltica* were either swimming (43%) or stayed on the artificial *F. lumbricalis* (38%) (ANOVA, $p < 0.001$). There was no significant difference in the habitat preference between the live and artificial *F. vesiculosus* (10% and 6%, respectively; Fig. 5).

In the feeding experiment, *I. baltica* practically only consumed *P. littoralis*. The grazing rate of *I. baltica* was on average (± SE) 0.332 ± 0.077 g dry weight alga per gram *I. baltica* per day. The average daily production of *P. littoralis* was 2.0% ± 0.1% based on dry weight. Taking into account the initial weight of the alga and the density of isopods, the grazing pressure of *I. baltica* on *P. littoralis* was 400% of the algal production. Also the net production rate of *F. vesiculosus* exceeded its consumption rate by the isopods. The average daily production of *F. vesiculosus* was 0.6% ± 0.1% based on dry weight.

Discussion

We found that *Idotea baltica* preferred *Pilayella littoralis* and *Fucus vesiculosus* as habitat over the other studied algal species. The robust, flat thalli of fucoids provided the isopod mainly shelter, but was of little value as food. The epiphytic macroalga *P. littoralis*

significantly added to the habitat quality of *F. vesiculosus*, indicating that *P. littoralis* was more attractive for *I. baltica* site than *F. vesiculosus*. This was also supported by the feeding choice experiment, which showed that *P. littoralis* constituted an essential part of the diet of the isopod *I. baltica*.

Previous studies have suggested that the habitat choice of isopods is mainly a function of algal morphology (e.g. Nicotri, 1980; Hacker & Madin, 1991). Despite a relatively low food value, *I. baltica* is attracted to large, tough, branched algae to which the dorsiventrally flattened animal body is well adapted. However, recent studies have demonstrated that grazers prefer to feed on filamentous algae (Shacklock & Doyle, 1983; D'Antonio, 1985; Worm & Sommer, 2000). Hence epiphytic food resources should be the prime factor to determine the presence of idoteids in macrovegetation (Boström & Mattila, 1999; Pavia et al., 1999). These findings correspond to field observations of positive relationships between epiphyte load and grazer density (Kotta et al., 2000; Worm & Sommer, 2000).

It is likely that the increasing epiphyte load in recent decades has triggered a substantial shift in the habitat selection of *I. baltica*. In less eutrophicated conditions, with only few epiphytes present, *I. baltica* seems to select canopy-forming macroalga to increase protection from predators (Stoner, 1980; Main, 1987). With an increasing epiphyte load, *I. baltica* is attracted to fully overgrown macrovegetation (Kotta et al., 2000; Worm & Sommer, 2000), but also to free-floating macroalgal mats consisting of the ephemeral algae (Norkko et al., 2000). This selection may be advantageous to isopods as fish seem to avoid biotopes dominated by ephemeral algae (Salemaa, 1979, 1987). Following the bloom of ephemeral algae the density of *I. baltica* has notably increased (Salemaa, 1979; Kangas et al., 1982; Arrontes, 1990; Duffy, 1990; Kotta et al., 2000). Consequently, isopods may escape predation pressure, which would explain their occurrence in more exposed microhabitat.

In the present study we found that *I. baltica* was highly selective in its food choice of epiphytic macroalgae. Compared to *P. littoralis*, *C. glomerata* significantly reduced the habitat value for *I. baltica*. Live *C. glomerata* has thick cell walls and is likely to be resistant to herbivory (Birch et al., 1983; Gabrielson et al., 1983; Paalme et al., 2002). Furthermore, adults *I. baltica* did not seem to be well adapted to crawl along *C. glomerata*; in some cases they were observed to become trapped into the filaments and died there.

During the experiments, *P. littoralis* was in an early stage of decomposition in the study area during the experiment (Paalme et al., 2002). The decomposing algae probably had less resistant cell walls and high concentrations of nitrogen and phosphorus, which makes the alga more attractive to mesoherbivores (Mann, 1988). In the light of high selectivity of *I. baltica*, the generally found poor correlation between epiphyte load and isopod density (Nicotri, 1980; Pavia et al., 1999) might be explained by an unfavourable species composition of the epiphytes. Seasonal changes in epiphyte species composition may further mask a relationship.

In the habitat choice experiment it was found that *I. baltica* preferred less robust algae than *F. vesiculosus*. Following the mass bloom of *P. littoralis* and the decline of *F. vesiculosus* in the northern Baltic Sea (Haahtela, 1981; Kangas et al., 1982; Salemaa, 1987), the majority of *I. baltica* switched into an alternative substrate, i.e. *Furcellaria lumbricalis* (Kotta et al., 2000). As compared to *F. vesiculosus*, this alga has a finer thallus. However, under less eutrophicated conditions *F. lumbricalis* has a low coverage of epiphytes and food availability for *I. baltica* is low

The conclusion of this study is that under more eutrophicated conditions the habitat choice of *I. baltica* is mainly due to the attractiveness of the algae as a food. It is likely that when the coverage of epiphytes is low (little eutrophication), and the active search for food considerably increases the risk to be eaten by predators, the habitat choice of isopod is more determined by the structural characteristics of the substrate offering the best protection against the predator. If epiphytes are effectively removed in eutrophicated conditions, *I. baltica* may have a positive effect on perennial macroalgae (Brawley & Adey, 1981; Robertson & Lucas, 1983; Brawley & Fei, 1987; Williams & Seed, 1992; Jernakoff & Nielsen, 1996). Thus, isopods act as stabilisers of the macroalgal communities by compensating nutrient effects on ephemeral algal growth (Neckles et al., 1993; Williams & Ruckelshaus, 1993; Jernakoff et al., 1996).

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Blue mussels (*Mytilus edulis*) in the diet of roach (*Rutilus rutilus*) in outer archipelago areas of the western Gulf of Finland, Baltic Sea

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Key words: blue mussel, roach, Baltic Sea, predation, diet analysis

Abstract

The blue mussel (*Mytilus edulis*) is one of the key species in the Baltic Sea ecosystem and it is living at the edge of its range in the western Gulf of Finland. Roach (*Rutilus rutilus*) is a freshwater fish species that has benefited from recent coastal eutrophication and is at present highly abundant in the outer archipelago of the Gulf of Finland. In 2000 and 2001, a total of 516 roach were sampled for diet analysis in three study areas. Shelled molluscs formed over 95% of the diet of roach, blue mussels being the dominant single species. The proportion of this species in the food of roach in the three study areas ranged between 38–61% for smaller roach (<225 mm) and 39–85% for larger (>225 mm) roach, indicating that blue mussel is a highly important food source for roach in outer archipelago areas of the western Gulf of Finland, in contrast to reports from other parts of the northern Baltic Sea. The scarcity of large blue mussels in mussel beds in the easternmost study area was reflected in the lower proportion of blue mussels in the diet of larger roach. However, the growth of roach was not affected by the availability of blue mussels. The twofold differences observed in the annual growth of roach between warm and cold years demonstrated that temperature is an important factor controlling the growth of roach in the western Gulf of Finland.

Introduction

Roach (*Rutilus rutilus*) is a freshwater fish species common in coastal waters of the northern Baltic Sea (Lappalainen et al., 2000). In temperate lakes, it is a typical food generalist, feeding on detritus and plant material as well as on zooplankton and zoobenthos (e.g. Persson, 1983; Horppila, 1994). Also, in coastal bay areas roach has been found to be omnivorous, feeding on both vegetable and animal food sources (Mattila & Bonsdorff, 1988). Stomach samples taken from roach in the Tvärminne archipelago, in the westernmost Gulf of Finland, have shown that mussels, blue mussels in particular, can dominate the diet of roach (Rask, 1989; Lappalainen et al., 2001). Blue mussel (*Mytilus edulis*) is one of the key species in the Baltic Sea ecosystem (Kautsky, 1981). It is a marine species, living at the edge of its range in the Gulf of Finland (Westerbom et al., 2002). It has long been considered that predation pressure on the dense northern Baltic blue mussel beds is low or almost lacking (Kautsky, 1981; Littorin & Gilek, 1999; Reimer & Harms-Ringdahl, 2001). In the Gulf of Finland, however, the mean size of blue mussels has declined significantly during the 1990s (Öst & Kilpi, 1997). The underlying reason have been proposed to be a decrease in salinity during recent decades, but it has also been suggested that predation by eiders (*Somateria mollissima*) has had local impacts on blue mussel populations by decreasing the proportion of larger individuals (Öst & Kilpi, 1997).

Roach has benefited from recent coastal eutrophication (Bonsdorff et al., 1997; Lappalainen et al., 2001) and is currently the most abundant species in the catch

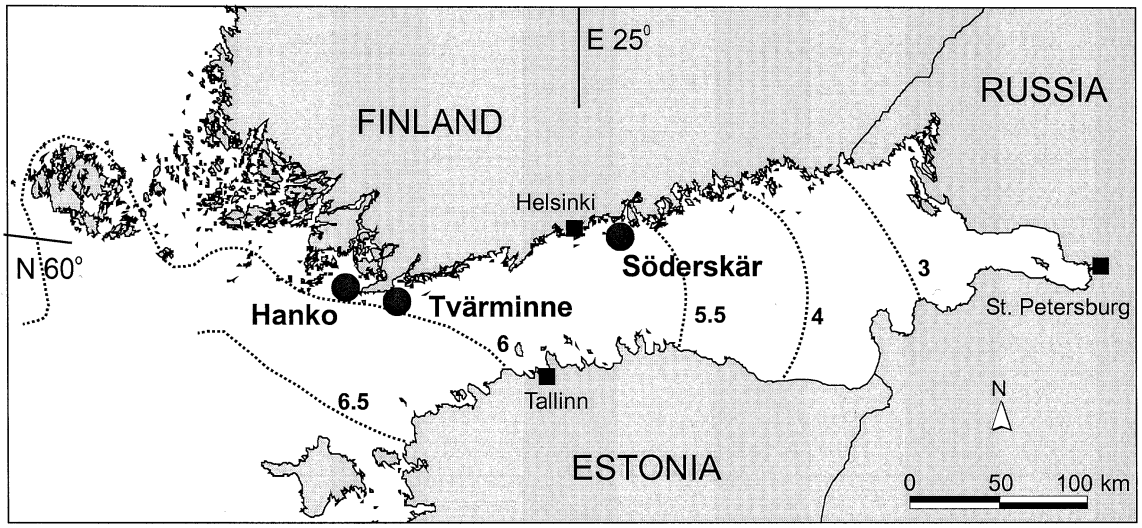

Figure 1. Location of study areas. The approximate isohalines (dotted lines), reflecting conditions in the open sea, are also given.

of gill net test fishing, even in the outer archipelago (Lappalainen et al., 2000), where the main habitats for blue mussels are found. This has raised the question of the potential effects of roach predation on local blue mussel populations in the western Gulf of Finland, where blue mussels live under conditions approaching their lower salinity tolerance. Low salinity levels cause slow growth and small maximum size for blue mussels (Kautsky et al., 1990). In the late 1990s, the blue mussel populations in the Gulf of Finland were dominated by small individuals, shell lengths being 2–10 mm (Westerbom et al., 2002). The majority of the mussels within the study area are potential prey for roach, which can eat mussels exceeding 15 mm (Prejs et al., 1990, Westerbom, unpublished data).

Our main objective was to quantify the diet of roach in the outer archipelago of the western Gulf of Finland. Special attention was paid on the role of blue mussels in the diet of roach. Our three study areas were located in regions with marked variations in the size structure and abundance of blue mussel populations, median size of blue mussels being small in the eastern part and larger towards the westernmost site (see Westerbom et al., 2002). As growth usually is considered to be a good indicator of the feeding conditions of fish, comparisons of roach growth were included in this study.

Materials and methods

The three study areas (Fig. 1) were all situated in the outer archipelago where rocky and sandy bottoms predominate. Surface water salinity decreases from 6–7 psu in the westernmost area to approximately 5–6 psu in the easternmost study area, which is close to the geographical limit of the blue mussel. The ranges of three other common mussel species, Baltic tellin (*Macoma balthica*), brackish water cockle (*Cerastoderma glaucum*) and sand gaper (*Mya arenaria*) extend further east, the Baltic tellin being common almost throughout the entire Gulf of Finland (Lassig, 1965). Annual variations in seawater temperatures are high, the surface water temperature varying from below freezing point during winter to, above 20 °C in most summers.

The sampling of roach for dietary analyses was done with gill nets (mesh sizes 12–60 mm) during the summer 2000. The nets were set on the bottom, usually before dusk, and hauled in the next morning. To terminate the activities of the alimentary canal, crushed ice was used to cool the fish. After capture, the fish were measured, weighed and sexed when possible, and their digestive tracts were removed and preserved in ethanol. At Tvärminne, the sampling was conducted at two sites (3 km apart) during two periods (June and August) to allow some spatial and temporal comparisons to be made. The total number of roach sampled was 239. In the other two areas, samples were taken in August at a number (5–7) of sites. The total number of fish sampled at Hanko was 159 and at Söderskär

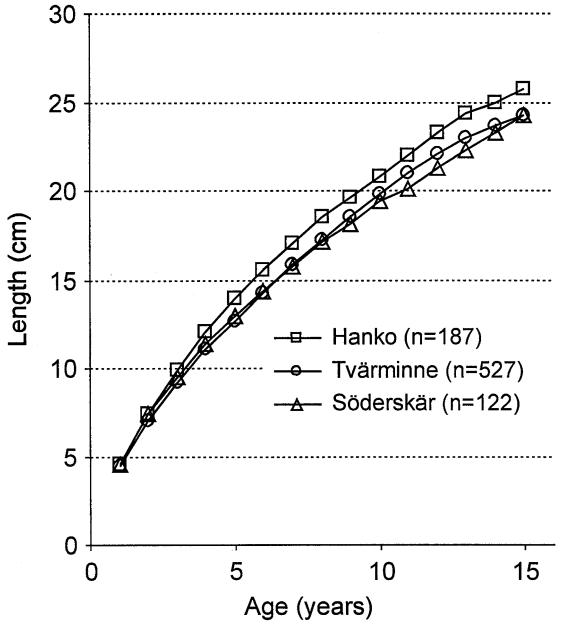

Figure 2. Back calculated length at various ages for roach in the three study areas.

118. Since only small roach were caught at Hanko, the data from this area were supplemented by catching 15 large roach in September 2001. The content of the anterior third of the gut was analysed under a binocular microscope. The volume proportions of different food items, estimated visually, were used to characterize the diets. Thereafter, a non-parametric Kruskal–Wallis test was applied for statistical testing. In addition, the frequencies of occurrence of different food items were counted. Fish with empty guts (22% at Hanko, 31% at Tvärminne and 40% at Söderskär) were excluded from the calculations.

The growth rate of roach was back-calculated from chleithrums according to the formula of Monastyrsky (see Bagenal & Tech, 1978). Some roach were concurrently sampled for other purposes from the two westernmost areas, and these individuals were also included in the growth analysis. The growth data collected from Tvärminne in 2000 were used to compare the annual growth of roach of different ages during the previous three years. Differences in growth patterns were tested by ANOVA and Scheffe's multiple-comparison tests.

Table 1. Volume percentage composition of the diet of roach in the three study areas in samples taken in June–August. A Kruskal–Wallis test was applied for statistical testing.

Food item	Hanko	Tvärminne	Söderskär	*p*-value
(a) Roach length <225 mm				
Mytilus	38	77	61	<0.001***
Macoma	0	1	0	<0.443 ns
Cerastoderma	1	0	10	<0.006**
Mya	0	0	0	–
Hydrobia	54	20	11	<0.001***
Theodoxus	1	2	12	<0.001***
Other Gastropoda	1	0	1	
Crustacean	4	0	5	
N	122	94	32	
(b) Roach length >225 mm				
Mytilus	63	85	39	<0.001***
Macoma	10	8	6	<0.776 ns
Cerastoderma	1	0	27	<0.001***
Mya	0	1	0	–
Hydrobia	16	5	12	<0.001***
Theodoxus	9	0	8	<0.001***
Other Gastropoda	1	0	4	
Crustacean	0	0	4	
N	15	72	39	

Results

Shelled molluscs formed over 95% of the diet of the sampled roach. The total volume proportion of mussels in the food of roach in the three study areas was 39–78% for smaller roach (<225 mm) and 74–93% for larger roach (Table 1). Snails (*Hydrobia* and *Theodoxus*) were also important food items, especially for small roach. Both the frequency of occurrence and the volume proportions showed that the blue mussel was clearly the dominant species in the diet of roach in all areas (Tables 1 and 2). The Baltic tellin had some importance for larger roach in all areas (6–10% of the food), but the brackish water cockle was an important food item for roach only in the easternmost study area, Söderskär (Table 1). In our study area, the sand gaper had no importance as a food item for roach. In addition to shelled molluscs, some crustacean were found in the diet samples, but plant or detritus items were only occasionally detected and the share of this material remained very low, <1%.

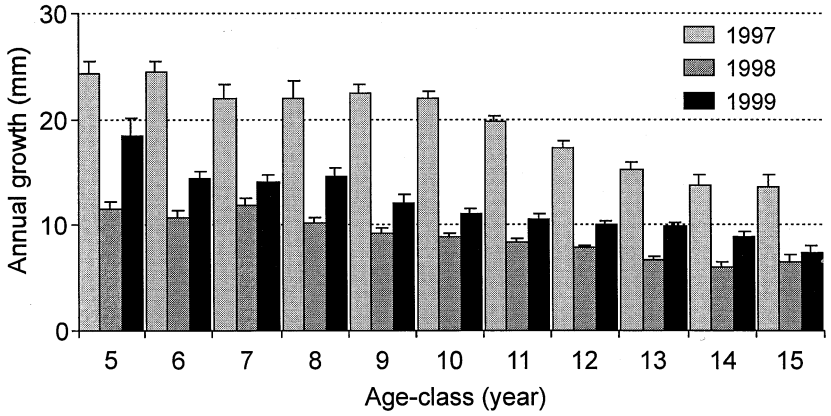

Figure 3. Annual growth (mean ± SE) of roach at Tvärminne in 1997–1999. The number of samples in single columns varies between 13 and 76.

In the westernmost study area, Hanko, the proportion of blue mussels was higher in the diet of larger roach than in that of smaller roach, although the difference was only marginally significant (Kruskal–Wallis test, $p < 0.057$). The proportion of blue mussels in the diet of roach was generally highest at Tvärminne, where the mussels were clearly more attractive to larger than to smaller roach ($p < 0.018$). At Söderskär, however, the opposite was found, as the proportion of blue mussels was higher in the diet of smaller roach than in that of larger roach ($p < 0.033$).

Comparisons at Tvärminne did not reveal any clear differences in the proportions of blue mussels in the diet of roach between the two sites (77% and 86%, Kruskal–Wallis test, $p < 0.864$). Similarly, no differences were found in the proportions of blue mussels in the diet of roach between the two sampling months, 82% in June and 79% in August ($p < 0.528$).

Spatially, the growth of roach was faster in the Hanko area than in the other two areas (Fig. 2), the differences in mean lengths between Hanko and the other areas being significant (ANOVA, Scheffe's test, $p < 0.05$) for age-classes of 4–13 years. Inter-annually, the mean annual growth of roach at Tvärminne was approximately twice as high during the warm growing season of 1997 as it was during the colder growing seasons of 1998 and 1999 (Fig. 3). The differences in the means between 1997 and the other two years were statistically significant (ANOVA, Scheffe's test, $p < 0.001$) for all age-classes studied (5–15 years). The differences between 1998 and 1999 were statistically significant (ANOVA, Scheffe's test, $p < 0.05$) for age-classes 5-, 6-, 8-, 11-, 12- and 13-years.

Discussion

Our results clearly demonstrated that roach commonly feed on blue mussels, not only in the Tvärminne area (see Rask, 1989) but also elsewhere in the archipelago of the western Gulf of Finland. The blue mussel is the only non-burrower among the four common mussel species. This, together with its high abundance in the rocky littoral, is a likely reason for the high dominance of blue mussels in the diet of roach. The diet of roach in our study differed markedly from that reported from other parts of the northern Baltic Sea where blue mussels occur more sparsely, or are totally lacking. In the Luleå archipelago of the Bothnian Bay, the northernmost part of the Baltic Sea, isopoda (*Saduria entomon*), gastropods and plant matter were important food items for roach (Hansson, 1984). In a coastal bay area in the Åland archipelago, SW Finland, molluscs were important for roach, but blue mussels were not among the most common prey species (Mattila & Bonsdorff, 1988). In temperate lakes, mussels do not generally seem to play an important role in the diet of roach (see Horppila, 1994; Tolonen et al., 2000).

The occurrence of blue mussels in our study areas varies considerably from the more saline west to the less saline east (Westerbom et al., 2002). The mean biomass of blue mussels in mussel beds during the late 1990s was 55 g m^{-2} (shell free dry weight) in the westernmost Hanko area and 13 g m^{-2} in the Tvärminne area but only 6 g m^{-2} in the Söderskär area. Similarly, the proportion of individuals longer than 10 mm decreased from west to east, being 25%, 2% and 0.1%, respectively (Westerbom et al., 2002). Larger (>225 mm) roach prefer blue mussels with a shell length of 10–18 mm (Westerbom, unpublished

Table 2. Frequency of occurrence of food items in guts of roach in the three study areas in samples taken in June–August.

Food item	Hanko	Tvärminne	Söderskär
Bivalvia			
Mytilus	67	91	76
Macoma	2	7	6
Cerastoderma	4	4	32
Mya	0	1	0
Gastropoda			
Theodoxus	7	4	33
Hydrobia	73	31	41
Bithynia	9	3	1
Lymnaea	2	1	6
Others			
Amphipoda	6	0	4
Isopoda	3	0	6
Balanus	2	5	10
Nereis	0	0	1
N	136	166	71

data). We suggest that the small number of blue mussels exceeding 10 mm in length in the Söderskär area was the main reason for the low proportion of blue mussels, and the higher contribution of other mussel species and snails in the diet of larger roach in that area. This is, however, not reflected in the growth of roach, as growth of the species in Söderskär area was similar to that at Tvärminne.

Roach is a typical warm-water species, and the lower temperature thresholds for cyprinid growth in usually between 12–15 °C (Mann, 1991). The relationship between temperature and growth was clearly evident in the comparison of roach growth during 1997–1999 at Tvärminne. The annual growth of roach in 1997, when the summer was very warm, was up to twice as high as in the 'cold year' of 1998. Water temperatures have been measured at 10-day intervals in the Tvärminne area. According to these data, the estimated number of days when the water temperature exceeded 15 °C at a depth of 3 m was 100 in 1997, 60 in 1999 but only 10 in 1998 (Tvärminne Zoological Station, unpublished data). Comparative temperature-related differences in growth have been reported from the Baltic Sea area, for example, for perch (Neuman, 1976). The high differences observed in the growth of roach between years suggest that also the food consumption of the species in the Tvärminne area may vary considerably between warm and cold years.

Roach is at present highly abundant in the entire archipelago of the Gulf of Finland (Lappalainen et al., 2000) and, as our results confirmed, the blue mussel is the dominant species in the diet of roach in the outer archipelago areas. On the basis of our results, we suggest that roach predation may affect the dynamics of blue mussel populations, especially in the central parts of the Gulf of Finland, where growth of mussels is slow, larger mussels are few (Westerbom et al., 2002) and mortality is high (Westerbom et al., unpubl.). This is most probably the case at Söderskär, the easternmost area with the most extreme salinity conditions contributing to the lowest biomasses of blue mussels. Rutkowski (1994) has shown by cage experiments conducted in a mesotrophic Polish lake that predation by roach strongly reduces the abundance of large (10–15 mm long) individuals of the zebra mussel (*Dreissena polymorpha*). Experimental setups are also needed in our study areas to substantiate the effects of increased roach predation on local blue mussel populations.

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The effects of turbidity and light intensity on the consumption of mysids by juvenile perch (*Perca fluviatilis* L.)

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Abstract

Nursery areas of perch, *Perca fluviatilis* L., in the shallow coastal areas of the Baltic Sea are affected by increased water turbidity due to the ongoing eutrophication and other physical disturbances. Visually feeding fish, such as perch, which depend on clear water and good light conditions for effective foraging are often negatively affected by turbidity. We tested the following hypothesis: in laboratory experiments increased turbidity combined with decreased light intensity impairs the foraging efficiency of juvenile perch. Consumption of the mysid shrimp, *Neomysis integer* Leach, by 0+ perch (4–5.2 cm) and 1+ perch (6.1-7.7 cm) was tested at four levels of turbidity (1, 10, 20 and 30 NTU = Nephelometric Turbidity Units) combined with three light intensities imitating daylight (64–71 μmol m^{-2} s^{-1}), twilight (1.0–1.2 μmol m^{-2} s^{-1}) and night (complete darkness). There were no significant reductions in the consumption of mysids by 0+ and 1+ perch with increased turbidity and decreased light intensity in 3 h trials. The consumption of mysids by 0+ perch decreased slightly, although not significantly, with increasing turbidity in 1.5 h trial. This indicates that there are compensatory factors (e.g. increased activity of perch, increased prey encounter, reduced anti-predator behaviour of prey, altered contrast of prey) acting on reduced visual ability of juvenile perch due to increased turbidity and low illumination.

Introduction

Warm water fish, such as perch, use the most shallow and sheltered parts of the coastal zone in the Baltic Sea as nursery areas. These areas are important for first year growth and survival due to higher water temperature (Karås, 1996a,b) and habitat complexity compared to deeper areas (Rossier et al., 1996, and references therein). Due to the ongoing eutrophication in the coastal waters of the Baltic Sea, the nutrient load followed by phytoplankton density have increased with large-scale effects on aquatic communities (Elmgren, 1989; Jumppanen & Mattila, 1994; Bonsdorff et al., 1997). One of the consequences, especially in shallow and sheltered areas, is changes in the physical environment through increased turbidity and decreased transparency of the water due to higher amount of organic particles (Bonsdorff et al., 1997). Human activities, such as dredging, boat-traffic and other mechanical disturbances in shallow recruitment areas also cause turbation through resuspension of sediment particles (Degerman & Rosenberg, 1981; Blomqvist, 1982).

Suspended particles scatter and absorb light and therefore reduce visibility in the water column (Lythgoe, 1979). Increased turbidity may negatively affect feeding success of visually feeding fish by decreasing reactive distance (Vinyard & O'Brien, 1976; Gregory & Northcote, 1993; Utne, 1997) and reducing visual range, i.e. reducing the volume of water searched (Aksnes & Giske, 1993). Water temperature and feeding success affect growth in the early life of fishes and thus influence survival, year-class strength, and recruitment (Bannister et al., 1974; Houde, 1987; Karås, 1996a). Several experimental investigations have demonstrated negative effects of turbidity on the foraging success of numerous fish species (Johnston & Wildish, 1982; Breitburg, 1988; Miner & Stein,

1993; Gregory & Levings, 1998). However, some studies show no effects of turbidity on foraging rate (Breitburg, 1988; Vandenbyllaardt et al., 1991; Reid et al., 1999) or show enhanced foraging with increasing turbidity (Boehlert & Morgan, 1985; Gregory & Northcote, 1993). Little is known on the combined effects of turbidity and light on juvenile fish predation (Grecay & Targett, 1996), and no studies are done on juvenile perch.

After hatching in the littoral in spring, perch larvae migrate and spend about 1–2 months in the pelagic zone where they forage on zooplankton. Thereafter they return to the littoral zone, stay among submerged macrophytes and start feeding on macroinvertebrates (Wang & Eckmann, 1994). In Baltic coastal areas 0+ perch at a length of 40–45 mm switch from a diet of zooplankton to larger invertebrates, such as insect larvae, mysids and other crustaceans. In eutrophic areas, this process is delayed and zooplankton is an important food object even at 60-70 mm length (Sandström & Karås, 2002). Perch are visual hunters and they are diurnally and crepuscurlarly active (Persson, 1983; Huusko et al., 1996; Imbrock et al., 1996; Sandström, 1999). Therefore, they depend on relatively good light conditions and clear water for effective foraging (Ali et al., 1977; Bergman, 1988; Diehl, 1988). Day length restricts the time per day when light is sufficient for consumption of prey by visually feeding fish that depend on good light conditions (Karås & Thoresson, 1992). Therefore, increased turbidity could have greatest effect at dawn and dusk when light intensity otherwise is low and perch are highly active (Westin & Aneer, 1987). In twilight, the light intensity, combined with an increased turbidity might decrease to a level too low for effective foraging and thus shorten the time daily available for foraging. Bergman (1988) found that perch greatly decreased feeding efficiency at very low light conditions. However, other results show that perch are active at very low light intensities (Craig, 1977). Even a slight reduction in total food consumption may affect growth rates of juvenile fish, and thus influence year-class strength and recruitment (Karås, 1996a, b).

In this study, we investigated the combined effects of turbidity and light intensity on the feeding efficiency by 0+ and 1+ perch in laboratory conditions. The mysid shrimp *Neomysis integer* was used as prey species. Our hypothesis stated that increased turbidity combined with decreasing light intensity would significantly reduce fish predation on mysids. In the turbidity and light intensity matrix, difference in predation efficiency between year-classes was also analysed. In order to investigate the influence of experimental time on the ability of juvenile perch to consume prey in turbid and low light conditions, two experimental times (1.5 h and 3 h) were tested for 0+ perch.

Materials and methods

The experiments on 1+ perch were conducted in July 2000 and the experiments on 0+ perch in August and September 2000 at Husö biological station on the Åland Islands, the northern Baltic Sea. Foraging was studied in 30 l aquaria (length 40 cm, width 30 cm, height 23 cm), which were wrapped into black plastic in order to standardize background colour in each aquarium. The entire experimental area was also enclosed in black plastic to isolate it from outside visual disturbances. In order to mimic vegetation, six green plastic ribbons (length 20 cm) were anchored to a mesh (86 ribbons m^{-2}) at the bottom of each aquarium. Two centimetres of sand (grain size $\leq$ 0.5 mm) covered the mesh.

Four different levels of turbidity (1, 10, 20 and 30 NTU) were tested on both age classes of perch. A turbidity of 3 NTU equals a Secchi-disc depth of approximately 4 m and 35 NTU a Secchi-disc depth of about 0.5 m. Turbidity levels in natural habitats of juvenile perch in the Baltic Sea archipelago areas vary between 1 and 45 NTU, but turbidity levels rarely exceed 30 NTU (A. Sandström, pers. commun.). Clay, taken from sea bottom (organic content 10.5 $\pm$ 0.3%), was used to create the different turbidity levels (except in 1 NTU treatments, which contained only filtered seawater). The clay was filtered through a 50 μm sieve, mixed into filtered seawater and maintained in suspension during the experiments by bubbling air in the aquarium. The turbidity was measured with a HACH Turbidimeter 2100 P. The turbidity levels remained satisfactory constant during the experiments and did not vary significantly among replicates or treatments (Minimum – Maximum values: 0.97–1.55 NTU, 10.25–11.41 NTU, 19.72–22.18 NTU, 28.60–31.35 NTU).

For both age classes of perch all four turbidity levels were tested at two light intensities imitating daylight respective dawn and dusk. All predation treatments were replicated five times and controls were used to evaluate non-predatory mortality of mysids. All treatments (inclusive the controls) were randomised among the experimental runs and within experi-

mental arrays. Light for the daylight treatments was provided by three fluorescent lamps placed above each aquarium (Philips fluotone 'TL' D 36/29). Two of the fluorescent lamps were placed 30 cm above the water surface and one fluorescent lamp was placed 126 cm above the water surface. In order to gain low light level only the fluorescent lamp placed 126 cm above the water surface was switched on and was wrapped in semi-transparent black plastic. Light intensity was measured at the surface of each aquarium before and after each trial with a LI-COR LI-190SA quantum sensor. Day light intensities at the surface of the aquaria ranged from 64 to 71 μmol m^{-2} s^{-1} (0.21–0.25 W m^{-2}) equal to ambient day light densities at 1 m depth in bright skies during summer. Low light intensities ranged from 1.0 to 1.2 μmol m^{-2} s^{-1} (13.6–15.1 W m^{-2}), which represent twilight light intensities after sunset in July. Exact illuminance values were not measured, but rough calculations showed that illuminance in the day light conditions was about 3100–3400 lx, and in the twilight conditions about 48–58 lx (Ryer, 1998). These calculations are based on the assumption that a third of the measured light wave spectrum (400–700 nm) was around 555 nm, which is the principal wave length for lux-measurements adapted for the human eye. Estimations of the irradiance are for the water surface and actual values were thus lower (depending on the turbidity level) in the water. The ability of 1+ perch to consume mysids in complete darkness was also tested in clear water and in 30 NTU. Based on results from pilot experiments a 3 h experimental time was chosen for both 0+ and 1+ perch. Later, 0+ perch were also tested in 1.5 h experiments. The daylight experiments were conducted at daytime (12:00 to 15:00) and the low light experiments at dusk (21:00 to 24:00, later in the autumn from 20:00 to 23:00) in order to follow the internal rhythm of the fish. The experiments in complete darkness were conducted at night (23:00 to 2:00).

Oxygen values in the treatments varied from 8.62 to 9.89 mg l^{-1}, pH from 8.02 to 8.64 and salinity from 5.21 to 5.54‰. The temperature fell during the experimental period, ranging from 17 to 19.9 °C for 1+ perch in July, from 14.7 to 18.8 °C for 0+ perch (3 h) in August and from 13.1 to 15.4 °C for 0+ perch (1.5 h) in September.

The mysids were always collected one day prior to respective trial. In the experiment with 1+ perch, the mean length of mysids was 15.0 ± 0.04 mm and for 0+ perch trials the mean length of mysids was 13.3 ± 0.06 mm. Juvenile perch were collected with a beach seine and kept in a 400 l aquarium with a flow-through system. The fish were mostly fed with live mysids, but also live chironomids and gammarids were used. The fish were allowed to acclimatize in aquarium conditions for two weeks prior to use in experiments. Mean length of 1+ perch was 67.3 ± 0.07 mm and mean weight was 2.4 ± 0.08 g. Mean length of 0+ perch in 3 h experiments was 47.5 ± 0.07 mm and the weight was 1.0 ± 0.06 g. Mean length of 0+ perch in 1.5 h experiments was 49.5 ± 0.06 mm and the weight was 1.1 ± 0.06 g. The fish were held without food for 35–40 h prior to each experiment. Each predation aquarium was divided in two compartments by a net wall. Two to three hours before the start of a trial, 50 mysids (1.7 ind l^{-1}) were added to one side of the aquarium in each replicate. In the other side of each aquarium, three randomly selected perches (0.1 ind l^{-1}) were allowed to acclimatize for one hour to the prevailing turbidity level and light intensity. Fish were run in groups of three in order to reproduce feeding conditions that resemble natural conditions. Availability of prey was adjusted so that perch should not get satiated even if they ate most of the prey during the experiment. Estimated maintenance ratios at our experimental temperatures for ca. 1 g respectively 2.5 g yellow perch (*Perca flavescens*) are about 0.4 respectively 0.96 g food g^{-1} d^{-1} (Kitchell et al., 1977). It was assumed that these ratios also apply on perch. Based on the ratios of basic maintenance, the food (prey biomass) introduced and evenly distributed among the fish (i.e. 16.7 prey per fish) in each trial would have provided the fish with 30 (for 1+ fish)-50 (for 0+ fish) % of the food needed per day. These values are theoretical maximum values. In pilot experiments (3 h duration), a single fish consumed at maximum 12 mysids in clear water treatments, i.e. 23% satiation of the daily need of food for basic metabolism. The experiment started when the net walls were gently removed and the fish were allowed to feed for the settled experimental time. The experiment was terminated by netting the fish. The length and weight of the fish were measured and the number of surviving mysids counted.

Two-way analysis of variance (ANOVA), after confirmation of normality and homogeneity, was used to determine whether turbidity and/or light intensity influenced prey consumption within respective year-classes. Two-way ANOVA was also used to determine eventual differences in prey survival in control treatments. Within levels of turbidity and light level, Student's *T*-test was used to detect differences between predation and control treatments. Three-way ANOVA

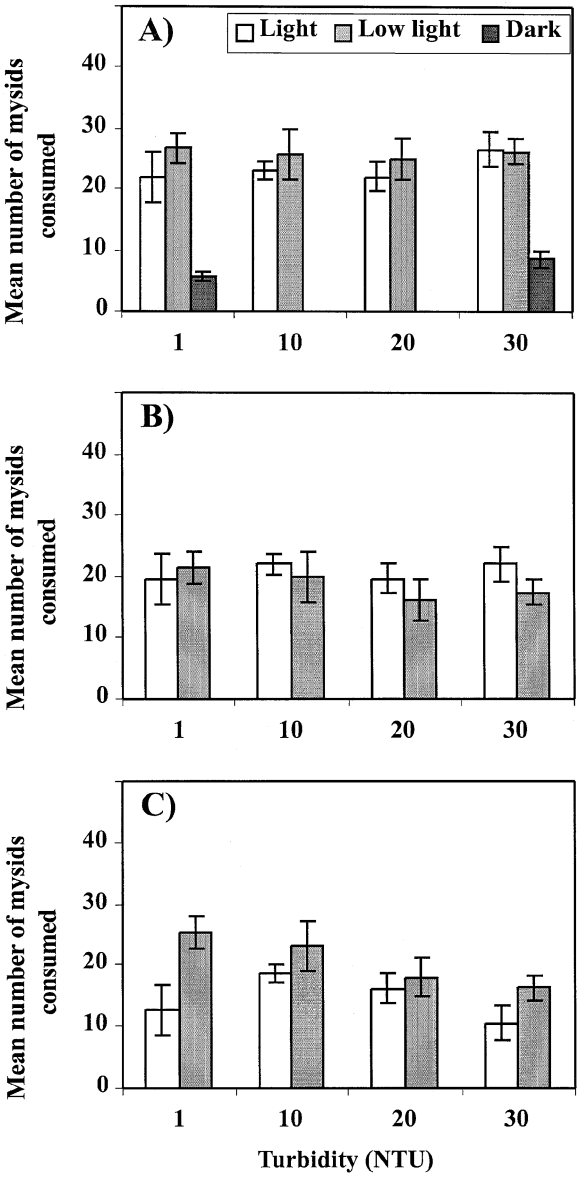

Figure 1. Effects of turbidity and light level on the number of mysids consumed by juvenile perch. Experimental time for 1+ perch was 3 h (A) and for 0+ perch 3 h (B) and $1\frac{1}{2}$ h (C). Consumption in darkness was tested only for 1+ perch. Each bar represents the mean of five replicates (± S.E).

was used to detect interactions among turbidity, light intensity and year-class (0+ and 1+ perch) on prey consumption. Three-way ANOVA was also used for detection of differences in prey consumption among turbidity, light intensity and experimental time (3 h and 1.5 h) for 0+ perch.

Table 1. Two-way ANOVA results using the number of mysids consumed at two light levels (light/low light) × four turbidity levels (1, 10, 20 and 30 NTU). In (a) three light levels (light/low light/dark). df = degrees of freedom, SS = sum of squares, * = significant at $P < 0.01$ and ** = significant at $P < 0.001$.

Source of variance	df	SS	*F*-value	*P*-value
(a) 1+ perch (3 h)				
Light level	2	2297.6	31.43	0.000**
Turbidity	3	65.9	0.60	0.618
Light level × turbidity	4	36.7	0.25	0.907
(b) 0+ perch (3 h)				
Light level	1	42.0	1.72	0.199
Turbidity	3	51.7	0.70	0.556
Light level × turbidity	3	61.9	0.84	0.480
(c) 0+ perch (1.5 h)				
Light level	1	384.4	10.28	0.003*
Turbidity	3	292.6	2.61	0.069
Light level × turbidity	3	165.8	1.48	0.239

Results

Turbidity/light level feeding experiments within year-classes

For 1+ perch, there was a significant reduction in foraging efficiency in darkness compared to day and low light level (Fig. 1a, Table 1a). For both 1+ perch and 0+ perch (experimental time 3 h) there were no differences in consumption of mysids over turbidity levels between day and dawn/dusk light intensities (Fig. 1a,b, Table 1a,b). When the experimental time was 1.5 h for 0+ perch, they consumed significantly more prey at low light level compared with day light level (Fig. 1c, Table 1c). There was a slight but not significant decrease in consumption of mysids with increasing turbidity. No significant interaction was found between light and turbidity (Table 1c). Survival of mysids in control treatments was high (94–100%) and there were no significant differences in the survival among turbidity levels within any level of light (Two-way ANOVA test, $p = 0.18$–0.85). For both year-classes, there were significant differences in survival of mysids within levels of turbidity and light level between predation and control treatments (Student's *T*-test, $p = 0.00$–0.037).

Table 2. Mean values (± standard error of the mean, SE) of 1+ and 0+ perch: mysid biomass ratios.

	1+ perch	0+ perch
Daylight		
1 NTU	7.10 ± 1.07	4.16 ± 0.19
10 NTU	6.70 ± 0.44	3.68 ± 0.40
20 NTU	7.01 ± 0.59	3.68 ± 0.17
30 NTU	5.90 ± 0.47	3.95 ± 0.48
Low light		
1 NTU	5.98 ± 0.60	4.12 ± 0.58
10 NTU	7.31 ± 2.02	4.41 ± 0.56
20 NTU	7.03 ± 0.64	4.67 ± 0.23
30 NTU	5.59 ± 0.46	4.87 ± 0.31

Turbidity/light intensity effects on foraging efficiency between year-classes

The mean weight of 1+ perch in the turbidity/light level treatments was 2.45 ± 0.08 g. The mean weight of mysids used in these experiments was 17.9 ± 0.1 mg. The mean weight of 0+ perch was 1.0 ± 0.06 g respectively 11.9 ± 0.1 mg of the mysids. This means that 0+ perch fed on larger prey than 1+ perch in proportion to their own weight. Therefore, it was not appropriate to directly compare the number of prey consumed between the two year-classes. Instead, we counted for both year-classes a fish biomass: mysid biomass ratio for each turbidity and light level combination (Table 2) and used these values in the analysis of variance. The temperature varied between the two year-class treatments (1+ perch: 18.5 ± 0.3 °C, 0+ perch: 16.9 ± 0.6 °C) and was therefore used as a covariate in the analysis. Only the main effect of year-class was significant (Table 3a) and showed that 0+ perch had consumed more biomass in proportion to their own weight than did 1+ perch. This effect was independent of turbidity and light intensity. Temperature had no significant effect on the consumption of mysid biomass (Table 3a).

Effects of turbidity and light intensity on feeding by 0+ perch at different experimental times

Mean number of prey consumed was used in the analysis. The temperature differed between 1.5 and 3 h experimental times and was thus used as a covariate in the analysis (1.5 h: 14.2 ± 0.3 °C, 3 h: 16.9 ± 0.6 °C). The main effects of experimental time, light intensity and turbidity had a slight but not significant influence on consumption of mysids (Table 3b). There was an interaction between experimental time and light intensity (Table 3b). When the experimental time was 1.5 h, the consumption of mysids by 0+ perch was significantly lower in daylight compared to low light level and to consumption in both light intensities in 3 h trial. Temperature had no significant effect on the consumption of mysids (Table 3b).

Discussion

The ability of 1+ perch to consume mysids in complete darkness was very low during a 3 h trial conducted at night. This is consistent with other results showing that perch are nocturnally inactive and that feeding rate during night is low (Diehl, 1988; Imbrock et al., 1996). The results of our study show that turbidities as high as 30 NTU combined with twilight light intensity do not reduce the ability of 0+ and 1+ perch to capture mysids in 3 h trials. The consumption of mysids by 0+ perch decreased slightly, although not significantly, with increasing turbidity in 1.5 h trial.

Several authors have recorded high activity and feeding by perch during twilight in clear lakes (Craig, 1977; Wang & Eckmann, 1994; Huusko et al., 1996; Imbrock et al., 1996), although perch are physiologically adapted to good light conditions (Bergman, 1988). In the 3 h experimental trials both 1+ and 0+ perch consumed slightly, although not significantly, more prey in low light compared to day light intensity in clear water. Perch in 1.5 h trial had a significantly higher feeding rate at low light level compared to higher light level (Fig. 1c). This may be due to increased activity during low light conditions. However, Bergman's results (1988) showed that perch decrease swimming speed and attack success with decreasing light intensity. It is surprising that 0+ and 1+ perch in our experiments were able to maintain their predation efficiency at such high turbidity levels combined with low light intensity. We initially hypothesized that low light levels resulting from increased turbidity during twilight light intensity would weaken the predation success for juvenile perch. Benfield & Minello (1996) showed that predation rate for gulf killifish, *Fundulus grandis* Baird and Girard, was unaffected by low light intensity, but was significantly lower in treatments with high turbidity where the light intensity was reduced to the same level as the low light treatment. They suggested that the influ-

Table 3. (A) Three-way ANOVA results using the values of fish: mysid biomass ratios at two light levels (light/low light) × four turbidity levels (1, 10, 20 and 30 NTU) × two year-classes. (B) Three-way ANOVA results using the number of mysids consumed at the same light and turbidity levels × two experimental times. Temperature was in both cases used as a covariate. df = degrees of freedom, SS = sum of squares, * = significant at $P < 0.01$ and ** = significant at $P < 0.001$.

Source of variance	df	SS	*F*-value	*P*-value
(a) 0+ perch vs 1+ perch (3 h)				
Temperature	1	0.01	0.006	0.938
Year-class	1	61.9	26.53	0.000**
Light level	1	0.9	0.39	0.534
Turbidity	3	2.9	0.41	0.749
Year-class × light level	1	2.9	1.22	0.273
Year-class × turbidity	3	7.1	1.02	0.390
Light level × turbidity	3	4.4	0.63	0.598
Year-class × light level × turbidity	3	0.9	0.13	0.943
(b) 0+ perch, 1.5 h vs 3 h				
Temperature	1	41.6	1.35	0.249
Experimental time	1	113.1	3.68	0.060
Light level	1	118.0	3.84	0.055
Turbidity	3	231.1	2.50	0.067
Exp. time × light level	1	340.5	11.07	0.001*
Exp. time × turbidity	3	94.8	1.03	0.387
Light level × turbidity	3	192.3	2.08	0.111
Exp. time × light level × turbidity	3	19.1	0.21	0.891

ence of suspended particles on predation rate was a consequence of light scattering and was not related to a decrease in light intensity. On the other hand, Vinyard & O'Brien (1976) showed that feeding success (measured as reactive distance) of visually feeding fish (bluegill, *Lepomis macrochirus*) was strongly decreased with increasing turbidity respective decreasing illumination. Effects of turbidity were consistent at all light levels (up to 215 lx), but they were enhanced as the illumination level decreased. Reactive distance in clear water was relatively long still at about 15 lx, and a drastic decrease occurred first when illumination decreased to about 10 lx. Our approximated illumination levels at the water surface were about 48–58 lx in the low light (twilight) treatment. At increased turbidity levels, the illumination was certainly lower than that in the water, but it may not have decreased to levels low enough to drastically affect predation efficiency of perch. Some studies have shown that the light threshold for visual feeding fish can be as low as 10^{-5}–10^{-2} lx (Blaxter, 1975). Such low levels were probably reached in our experiment only in the complete darkness treatment (Gal et al., 1999), where also the feeding activity was drastically reduced compared with the other treatments.

Studies on the effects of turbidity on foraging behaviour of different fish species have not been consistent. Reid et al. (1999) suggested these inconsistencies to be a result of three factors: changes in predator behaviour, changes in prey behaviour, and experimental design.

Several studies put forward that turbid environments may afford protection to young fish from their own predators (Gradall & Swenson, 1982; Gregory, 1993; Gregory & Northcote, 1993; Gregory & Levings, 1998). They can thus engage in activities that would be risky under clearer conditions, such as increased feeding activity (Gregory & Northcote, 1993; Gregory & Levings, 1998) and reduced cover-seeking behaviour (Gradall & Swenson, 1982; Gregory, 1993). When fishes increase their foraging activity the encounter rate with prey may increase (Vandenbyllaardt et al., 1991). This possibly will constitute a survival advantage, which obviates the negative effects

of reduced feeding and growth rates under turbid conditions (Grecay & Targett, 1996). Turbidity may function as a shelter from predation and increase activity and feeding in the same way as many fish species are active during twilight in order to avoid predation. However, the combination of high turbidity and low light level may visually affect perch to such an extent that it is unlikely that increased activity alone can explain the foraging behaviour of 0+ and 1+ perch in our experiments.

Prey behaviour may also be affected by turbidity (Gradall & Swenson, 1982; Gregory & Levings, 1996) and light level (Buskey, 2000). With increasing turbidity, predator-prey encounters will occur at shorter distances with the probability of escape by the prey ultimately being determined by the probability of detecting the predator before the predator detects them (Abrahams & Kattenfeld, 1997). Mysids try to avoid high light levels (Gal et al., 1999), which may have increased their vulnerability in day light treatments. At low light levels mysids increase their swimming speed (Buskey, 2000), which again may have increased the risk of encounter rate feeding at low light levels. There are several studies that report reduced anti-predator behaviour (e.g. schooling) with increasing turbidity (Gradall & Swenson, 1982; Gregory, 1993; Abrahams & Kattenfeld, 1997). Mysids perform aggregative behaviour where vision appears to play a very important role (Buskey, 2000). Mysids are potential prey to a wide range of fish species, and their survival may depend upon their ability to remain within schools and perform complex predator-avoidance behaviours in response to visually detected predators (O'Brien & Ritz, 1988). Turbidity may diminish the advantage of schooling behaviour in predator avoidance (Reid et al., 1999). However, we did not observe any distinct schooling behaviour by the mysids during clear water conditions, but it is possible that some predator-avoidance behaviours of mysids were reduced in turbid conditions compared to clear conditions. Combined with probably higher activity of perch in more turbid water with low illumination, this may have resulted in increased prey encounter rate and could compensate for the reduced visual ability for juvenile perch. Gregory & Levings (1998) suggested that any effects of turbidity on predation must be driven by its effects on the rate of encounter between predator and prey. In an aquarium, it is unlikely that encounter rates between highly mobile predators and prey will be appreciably reduced in all but very turbid conditions. Grecay & Targett (1996) claimed that encounter rate feeding is less efficient than visual feeding. Under such circumstances, feeding could become directly dependent on prey concentration. At low prey density, turbidity might affect prey capture to a higher extent (Breitburg, 1988). The capture of *N. integer* by 0+ perch is more affected by low prey density in turbid water compared to clear water (A. Sandström, pers. commun.). The equal feeding efficiency among all levels of turbidity at high and low light intensity in our experiments may have been due to the density of mysids used (1.7 mysids l^{-1}).

The foraging success of fish also depends on prey visibility. The visual contrast of prey is generally impaired in turbid environments (Johnston & Wildish, 1982; Aksnes & Giske, 1993). However, the visual contrast of an object can actually be enhanced in turbid conditions, depending on the type of prey, and affects positively the ability of a predator to detect prey (Boehlert & Morgan, 1985; Miner & Stein, 1993). Particles in the water scatter light in all directions and this could result in greater illumination of prey, which in turn can decrease transparency of the prey (Boehlert & Morgan, 1985). This may have been the case with *N. integer* in turbid water. Increased contrast of prey may counterbalance limitations of the visual field, but probably only up to some threshold level of turbidity where the decrease in visual volume will be more influential on consumption rates. The type of turbidity may also affect contrast of prey because different particles in the water (algae, organic particles, suspended clay) absorb and scatter light differently (Lythgoe, 1979). Therefore, effects observed in turbidity caused by clay respective algae are not easily comparable. However, no significant difference in consumption of *Bosmina longirostris* O.F. Müller by perch larvae could be found between algae and clay turbidities (A. Sandström, pers. commun.). This indicates that different suspended particles can affect the contrast of prey in the same way or that the effect of contrast is insignificant.

The equal feeding efficiency among all levels of turbidity in the 3 h trials may also have been a consequence of 0+ and 1+ perch having sufficient time to encounter prey and could have compensated for any reductions in foraging ability due to increased turbidity and low light conditions. On the other hand, food consumption in all treatments remained clearly lower than the daily energy based need of food in juvenile perch. At maximum, only 15 (1+ perch) to 25 (0+ perch) % of the maintenance level (Kitchell et al., 1977) was reached in any treatment, and in

the clear water treatments the fish were still actively searching for food at the time of termination. Fish behaviour could unfortunately not be observed in the turbid water treatments. Therefore, it seems unlikely that the experimental duration was too long and resulted in satiation of fish. There was a slight, although not significant, difference in the main effect of turbidity on consumption of mysids between 1.5 and 3 h experimental time for 0+ perch. The consumption by 0+ perch decreased with increasing turbidity in the $1\frac{1}{2}$ h trial. Vandenbyllaardt et al. (1991) and Grecay & Targett (1996) put forward that differences in foraging efficiency among turbidities can become evident if foraging time is limited.

0+ perch consumed significantly more mysid biomass in proportion to their own weight than did 1+ perch, but the difference in consumption was independent of turbidity and light intensity. Metamorphosed 0+ perch have developed similar visual apparatus as 1+ perch and hence both may be affected in the same way by a reduced visual volume. Feeding by larger fish is generally more affected by increased turbidity than that by smaller fish because the visual volume increases with the size of fish (Sandström, 1999, and references therein). However, larger perch have widely distributed feeding areas and the resource availability is usually good in such diverse habitats and could act as a compensatory factor for adult survival (Sandström & Karås, 2002). Therefore, growth and survival during the first year of perch life, when the juveniles are restricted to the most shallow and sheltered areas, often regulate population dynamics (Persson & Greenberg, 1990; Karås, 1996a).

In our study, juvenile perch were not poor feeders on mysids in low light conditions with high turbidity, indicating that there are compensatory factors (increased activity of perch, increased prey encounter, reduced anti-predator behaviours of mysids, altered contrasts of mysids) acting on reduced visual ability of juvenile perch. However, the compensatory factors surely depend on prey type (species, colour and size), density of prey and type of turbidity. Therefore, further studies are needed in order to clarify the effects of turbidity and low illumination on the visual ability and feeding efficiency by juvenile perch in the Baltic Sea.

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POPULATION STRUCTURE AND DYNAMICS

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The growth of pikeperch (*Sander lucioperca* L.) and perch (*Perca fluviatilis* L.) under different water temperature and salinity conditions in the Curonian Lagoon and Lithuanian coastal waters of the Baltic Sea

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Key words: salinity, temperature, pikeperch, perch, Baltic Sea, Curonian Lagoon

Abstract

Pikeperch and perch perform seasonal migrations between the Curonian Lagoon and the coastal waters of the Baltic Sea. The Curonian Lagoon is a freshwater basin, while salinity in the coastal waters varies between of 4.9–6.8 psu. In the Curonian Lagoon water temperature is generally higher than in the coastal waters. Field studies of growth and condition characteristics of pikeperch and perch were carried out in these water bodies with the aim to estimate growth differences of the two fish species under different salinity and temperature conditions. Additionally, an experimental study of the impact of salinity on the growth of perch young-of-the-year (YOY) was performed to test the hypothesis that a brackish environment positively influences percids. Field observations revealed that body length, condition factor, fatness coefficient and fat content in muscles were significantly higher in individuals inhabiting the cooler, brackish waters of the Baltic Sea than in individuals inhabiting the Curonian Lagoon. A positive effect of low salinity on growth was also established in the experimental study. Hence, the study results suggest that under certain temperature conditions, brackish waters beneficially affect the growth of pikeperch and perch.

Introduction

Pikeperch and perch are common in the inland waters of Europe and are usually considered typical freshwater fish species (Collette et al., 1977; Lehtonen et al., 1996). However, they also live in brackish waters (Neuman, 1979; Karås, 1996; Lehtonen et al., 1996). Pikeperch are locally abundant in coastal and littoral areas throughout the Baltic with the exception of the northernmost areas, exposed coasts and the most saline parts of the south-west Baltic Sea (Lehtonen et al., 1996). Adult pikeperch can tolerate relatively high salinities (9–10 psu) according to Lehtonen et al. (1996). Brown et al. (2001) found that pikeperch showed good tolerance of abrupt transfer to salinities of 8 and even 16 psu. Perch are widespread in the Baltic Sea and tolerate salinities up to 7–10 psu (Privolnev, 1970, cited in Thorpe, 1977; Lutz, 1972). Perch are found in the Aral Sea at 10 psu (Letitchevskij, 1946), but not in the Caspian Sea at 12 psu (Berg, 1965, cited in Thorpe, 1977).

Seasonal migrations between fresh and brackish waters in both species are well-documented (Gyllensten et al., 1985; Karås & Hudd, 1993; Skora, 1996). Perch populations migrate from brackish to freshwater habitats for spawning (Gyllensten et al., 1985; Karås & Hudd, 1993), suggesting that eggs and larvae may be less tolerant to brackish waters than adults. The importance of freshwater for spawning and nursery areas most likely could be attributed to warmer temperatures, better shelter in vegetation and more suitable food conditions (Erm, 1981; Urho et al., 1990; Karås & Hudd, 1993). However, it appears that freshwater is not the optimal environment for perch larvae development since it has been reported that adding 2 psu seawater to a freshwater basin can increase the survival rate of larvae (Bein & Ribi, 1994). Moreover, percids successfully spawn in the brackish waters of the Baltic

Sea (Karås & Hudd, 1993; Lehtonen et al., 1996; Neuman et al., 1996). However, salinities exceeding 9.6 psu and 4.75 psu have been reported limiting for perch and pikeperch larvae, respectively (Bein & Ribi, 1994; Lehtonen et al., 1996). Thus, the salinity tolerance of pikeperch and perch seems to depend on age.

It is widely accepted that the growth of pikeperch and perch is positively influenced by temperature (e.g. Karås & Neuman, 1981; Draulans et al., 1985; Elliott, 1985; Hudd et al., 1988; Wilkońska, 1988; Karås, 1990; Raat, 1990; Karås & Thoresson, 1992) and is food limited (e.g. Filuk, 1962; Popova & Sytina, 1977; Kjellman et al., 1996; Lehtonen et al., 1996). However, no studies on the effect of salinity on the individual characteristics of percids *in situ* have been published. Lind (1977) reported a slightly faster growth of pikeperch in the Baltic Sea compared to inland waters but suggested that this was associated with a greater food supply in brackish water.

Pikeperch and perch are common fish species in the Curonian Lagoon. Each year, after over-wintering and spawning in the lagoon, where the water is fresh and water temperature higher compared to the Lithuanian coastal zone of the Baltic Sea, the populations partly migrate to the brackish coastal waters of the Baltic Sea and return back to the lagoon in autumn. According to both commercial catches in the coastal zone, and test fishing in the Klaipėda strait and coastal zone, pikeperch begin to migrate to the sea abundantly in April and perch in May. Pikeperch and perch return back to the lagoon in September–October. In December–February coastal fishery and test fishing catches of perch and pikeperch are negligible. The advantages of after-spawning migrations to the colder Baltic Sea remain unexplored.

The objective of this study was to compare perch and pikeperch growth and condition characteristics under different salinity and water temperatures in the Baltic Sea and the Curonian Lagoon. It is possible that salinity will influence physiological processes of both young and adult fish as previously found for carp growth (larvae, YOY and adult fish) (Konstantinov & Martynova, 1990, 2000; Tiutiunik, 1961; Tchizhik & Ryzhnikov, 1973). Konstantinov & Martynova (2000) found a positive effect of brackish water (2 psu) on juveniles of several fresh water fish species (*Acipenser gueldenstaedti*, *Aristichthys nobilis*, *Ctenopharyngodon idella*, *Cyprinus carpio*). The hypothesis tested in the present paper is that a brackish environment would also beneficially affect growth of percids. With the aim to test this hypothesis, a survey of the perch YOY growth under different salinities was conducted.

Material and methods

Study area

The Curonian Lagoon is situated in the eastern part of the Baltic Sea (Fig. 1), with a narrow sand spit (0.5–4.0 km) separating the two. A connection is formed through the Klaipėda Strait. The total area of the lagoon is 1584 km^2, with a mean depth of 3.7 m. The Curonian Lagoon can be characterised as a freshwater basin since the average water level in the lagoon is 15 cm higher than that of the sea and hence penetration of seawater is rare. The salinity ranges from 0.03 psu in the southern part, up to 1.60 psu in the Klaipėda Strait. Stormy influxes may episodically increase salinity to 5–6 psu in the northern part of the lagoon (Olenin, 1996). The Curonian Lagoon is classified as eutrophic and seasonal water transparency (Secchi-disc depth) fluctuates in the range 0.35–2.0 m (I. Prochorova, pers. commun.). In comparison, the salinity in the Lithuanian coastal waters of the Baltic Sea varies from 4.9 to 6.8 psu (Dubra & Dubra, 1998) and water transparency varies from 1 m at the mouth of the Klaipėda Strait to 14 m in open areas of the sea (Vyšniauskas & Lesys, 1998).

For most of the year, water temperatures (measured twice daily at 0.5 m depth) differ significantly between the Curonian Lagoon and the Baltic Sea (A. Stankevičius, pers. commun.). Water was significantly warmer in the lagoon in March, April, May, June, July and August 1998–2000 (temperature difference (t): $t = 2.07$, $p < 0.05$; $t = 15.14$, $p < 0.01$; $t = 24.09$, $p < 0.01$; $t = 16.08$, $p < 0.01$; $t = 9.47$, $p < 0.01$; $t = 6.89$, $p < 0.01$, respectively). In September, water temperature did not differ significantly ($t = 1.89$, $p > 0.05$) (Fig. 2).

Field study

Material for the pikeperch and perch *in situ* growth study was collected during 1998–2000, in September when growth in length and accumulation of energy resources reached the yearly maximum. Difference in collecting time between the sites was 2–5 days. The growth study focused on pikeperch and perch individuals from the 1997 year class, since this generation dominated in both water bodies (individuals of the

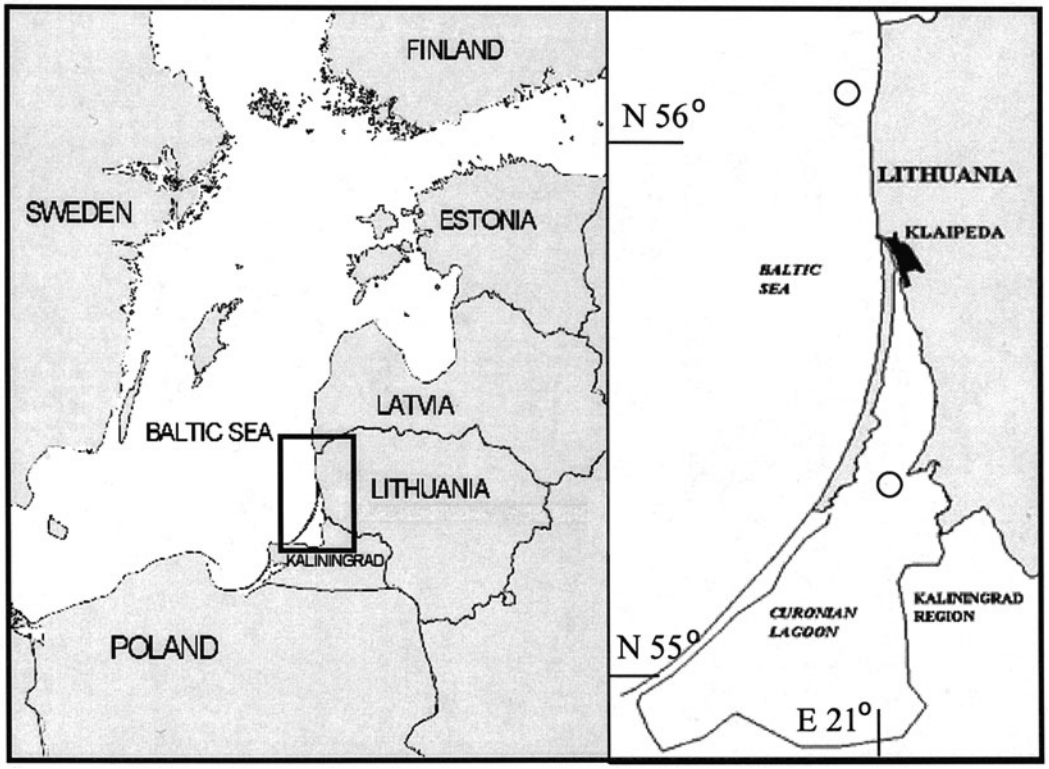

Figure 1. Study areas and material collecting sites (○).

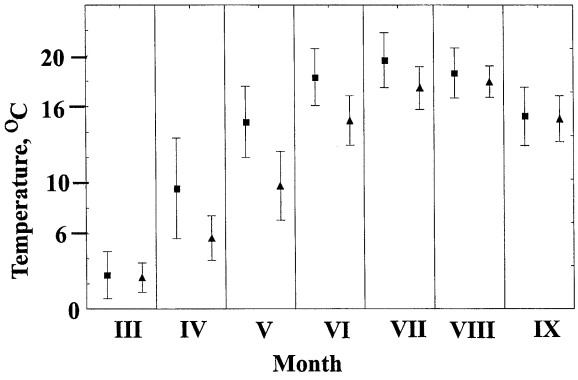

Figure 2. Water temperatures in the Curonian Lagoon (■) and the Baltic Sea (▲) during March–September 1998–2000 (vertical bars represent 1 SD).

other age groups were scarce). Fish were caught with gill nets (25, 30, 40, 45 and 50 mm mesh bars) set at 2.0–3.5 m and 2.5–4.5 m in the Curonian Lagoon and in the sea, respectively.

All pikeperch and perch were weighed (body weight in g = W, ±1.0 g) and measured (total length in cm = L, ± 0.5 cm) and condition factor (CF) and fatness coefficient (K_F) were calculated following Bagenal & Tesch (1978) and Bukelskis & Kublickas (1988): CF = $W/L^3 \times 100$ and $K_F = w/W \times 100$ (w = weight of fat on the intestines). These parameters were calculated for a specific pikeperch age group (3+). Since differences in the growth rate of perch for the same age group is considerable, the parameters were calculated for the same length, but different age perch individuals. Both sexes were combined in the calculations. Fat content (C_F) in muscle tissues was estimated using the Folch method (Bligh & Dyer, 1959; Lapin, 1973), and expressed as a percentage of wet weight. Pikeperch age was estimated from growth zones of scales, while opercular bones were used for perch (only females were aged). On the graphs of growth and condition parameters each point represents 50–100 individuals. For statistical analysis ANOVA, ANCOVA, Kruskal–Wallis, Levene's test and *t*-test were used.

Experimental study of growth

To evaluate the effect of salinity on percid growth, a survey of perch YOY growth under different salinity regimes was conducted. Perch YOY were collected by

seine netting at Ventė cape, a coastal area in the eastern part of the Curonian Lagoon (55° 21′ N, 21° 12′ E) in August 2001. After 5 days of acclimatisation in freshwater, the fish were randomly divided into 15 experimental groups. Each replicate group comprised 10 fish individuals, which were reared in identical 100 l glass aquaria under continuous aeration. The influence of salinity on perch growth was studied under three experimental salinity conditions: five aquaria contained water of 5 psu salinity, five water of 2 psu salinity and five contained freshwater. Salinity was achieved by dissolving sea salt in freshwater. One-third of the water in each aquarium was changed every second day. The duration of the experiment was three weeks. Water temperature was ambient in all aquaria and during the experimental period it decreased from 24.9 to 16.0 °C. Dissolved oxygen was measured at three-day intervals and ranged from 7.5 to 8.1 mg l^{-1}. All fish were fed with the same quantity of live *Chironomus* spp. larvae, weighed with an accuracy of 0.01 g. To ensure similar food consumption between replicates, each new portion of food was introduced immediately following consumption of the previous ration in all replicates. Total fish weight (± 0.01 g after excess water removed) for each replicate was measured prior to the beginning of the experiment and after one, two and three weeks. During the weighing procedure, individuals exhibiting disease symptoms were replaced with healthy individuals, which were reared in an identical fashion as experimental individuals in order to ensure the same density of fishes in all replicates. To assess differences in growth under different treatments, the weight increments of perch YOY groups of each replicate for each week were analysed using one-way repeated measures ANOVA.

Results

Growth and condition characteristics in situ

The body lengths for both percid species were significantly larger in the coastal waters of the Baltic Sea compared to the Curonian Lagoon (Fig. 3) (Pikeperch: 1+, $t = 9.89$, $p < 0.01$; 2+, $t = 2.05$, $p < 0.05$; 3+, $t = 2.54$, $p < 0.01$; Perch: 1+, $t = 6.28$, $p < 0.01$; 2+, $t = 11.33$, $p < 0.01$; 3+, $t = 85.97$, $p < 0.01$). Under experimental conditions and from field observations has been demonstrated that weight can increase, while the length remains constant (Karås, 1990; Karås & Neuman, 1981). Since the growth rate (length) was

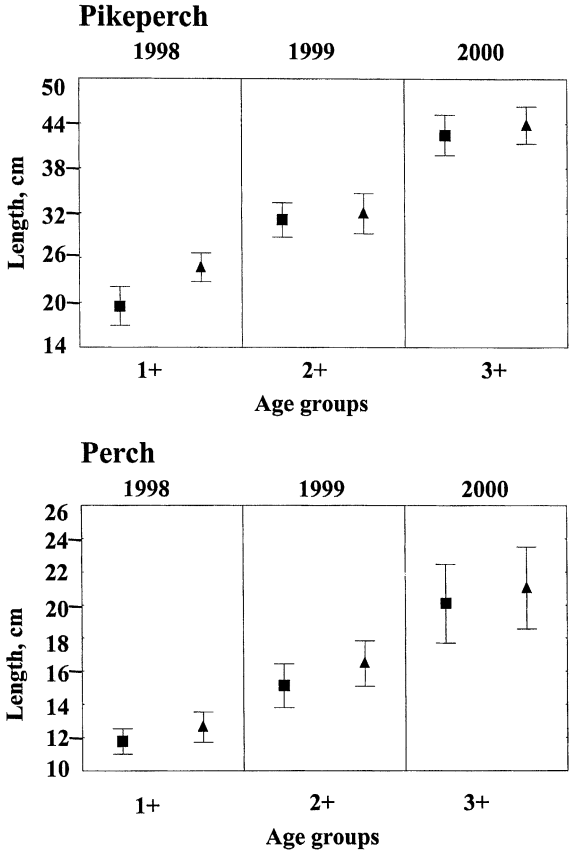

Figure 3. The length of pikeperch and perch of 1997 year class in the Curonian Lagoon (■) and the Baltic Sea (▲) during September of 1998–2000 (vertical bars represent 1 SD).

higher in the sea it could be presumed that definition of growth of studied fishes in the Curonian Lagoon is weight increase, while in the sea length increase predominates. Alternatively, pikeperch and perch in the lagoon may accumulate more energy resources (fat) in the muscles or on intestines and use this for growth in length later. To test the growth in weight and energy resource accumulation differences in the lagoon and the sea, CF, K_F and C_F in muscles and the effect of total body length on these parameters were evaluated (Fig. 4). In pikeperch, the effect of body length on CF was not significant (Curonian Lagoon: $F(1.81) = 0.15$, $p > 0.05$; Baltic Sea: $F(1.81) = 0.7$, $p > 0.05$). However, for pikeperch in the size range 40.5–52.0 cm, CF was significantly higher in fish from the Baltic Sea (Kruskal–Wallis test: H(1, N = 166) = 91.4, $p < 0.001$). In perch, the effect of body length on CF was weak but significant (Curonian Lagoon: $CF = 1.31 + 0.009L$, $F(1.74) = 9.5$, $p < 0.01$; Baltic Sea: $CF = 1.36 + 0.008L$, $F(1.74) = 4.02$,

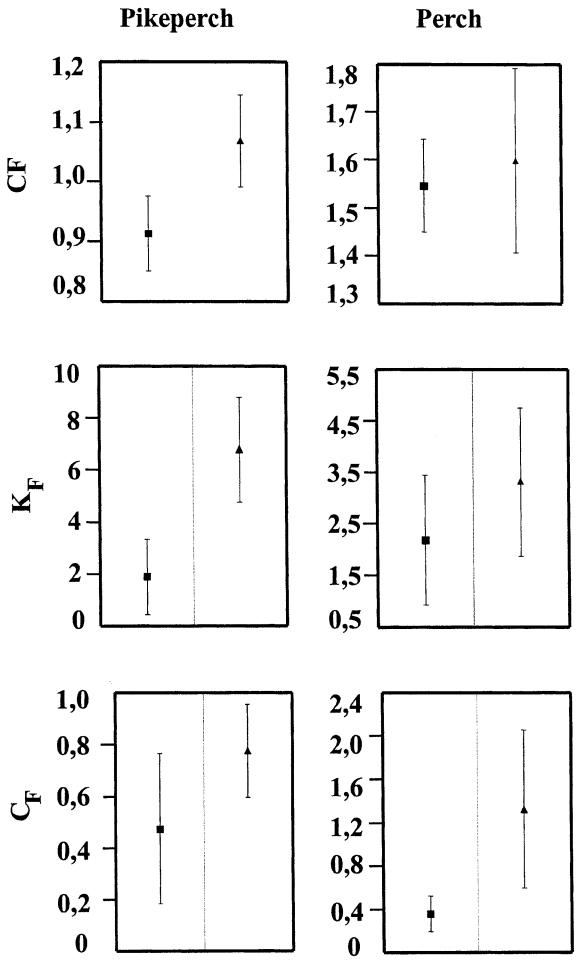

Figure 4. CF, K_F, C_F in muscles of the pikeperch (3+) and perch (same length groups) in the Curonian Lagoon (■) and the Baltic Sea (▲) in 2000 (vertical bars represent 1 SD).

$p < 0.05$). The variance of perch CF in the Baltic Sea was significantly higher than that in the Curonian Lagoon (Levene's test for homogeneity of variances: $F(1.150) = 38.7$, $p < 0.001$), therefore, a Kruskal–Wallis test was applied demonstrating significantly higher perch CF in the size range 14.5–39.0 cm in the sea ($H(1, N = 152) = 8.28$, $p < 0.01$).

In both pikeperch and perch the effect of body length on K_F was significant (Pikeperch: lagoon, $K_F = -9.44 + 0.25L$, $F(1.48) = 13.5$, $p < 0.001$; sea, $K_F = -24.05 + 0.64L$, $F(1.49) = 22.8$, $p < 0.001$. Perch: lagoon, $K_F = -2.62 + 0.16L$, $F(1.50) = 27.7$, $p < 0.001$; sea, $K_F = -1.98 + 0.17L$, $F(1.52) = 39.2$, $p < 0.001$). In pikeperch, increase of K_F with body length was significantly higher in the sea, while in perch this effect was not significant (Test of parallelism: pikeperch, $F(1.97) = 7.2$, $p < 0.01$; perch, $F(1.102) = 0.08$, $p > 0.05$). Differences between K_F of both pikeperch and perch from the lagoon and the sea were significant (Pikeperch: Kruskal–Wallis test, $H(1,N = 101) = 66.2$, $p < 0.001$). Perch: ANCOVA $M_{seffect} = 25.534$, $M_{serror} = 0.884$, $F = 28.8$, $p < 0.001$).

Table 1. Tukey's test for the experiment on perch YOY growth at different salinities (MS = mean of squares, df = degrees of freedom, F = test value in ANOVA, p = probability).

Source	MS	df	F	p
Salinity (S)	6.03033	2	12.12	< 0.001
Error	0.49736	12		
Week (W)	12.13630	2	19.15	< 0.001
S × W	0.28916	4	0.46	> 0.05
Error	0.63373	24		

The effect of body length on C_F was not significant (Pikeperch: lagoon, $F(1.48) = 3.88$, $p > 0.05$; sea, $F(1.49) = 3.02$, $p > 0.05$. Perch: Curonian Lagoon, $F(1.50) = 0.16$, $p > 0.05$; Baltic Sea, $F(1.52) = 1.19$, $p > 0.05$). Differences between C_F of pikeperch and perch from the lagoon and the sea were significant (Kruskal–Wallis test: pikeperch: $H(1, N = 101) = 23.7$, $p < 0.001$; perch, $H(1, N = 106) = 76.8$, $p < 0.001$).

Experimental study of the growth of perch

The growth of perch YOY under different salinities is shown in Fig. 5. The highest growth rate was observed in water of 5 psu salinity, while the lowest growth rate was found in freshwater. One-way repeated measures ANOVA revealed a significant effect of salinity on perch growth rate (Table 1). Differences between growth in brackish and freshwater were highly significant, while those between different salinities were not significant (Tukey's test: 5 psu vs. freshwater, $p < 0.01$; 2 psu vs. freshwater, $p < 0.01$; 5 psu vs. 2 psu, $p > 0.05$).

Discussion

Fish growth is associated with various individual characteristics. Karås & Neuman (1981) stated that if growth is defined as an increase in weight, then growth of perch may occur even at 4 °C, while if is defined

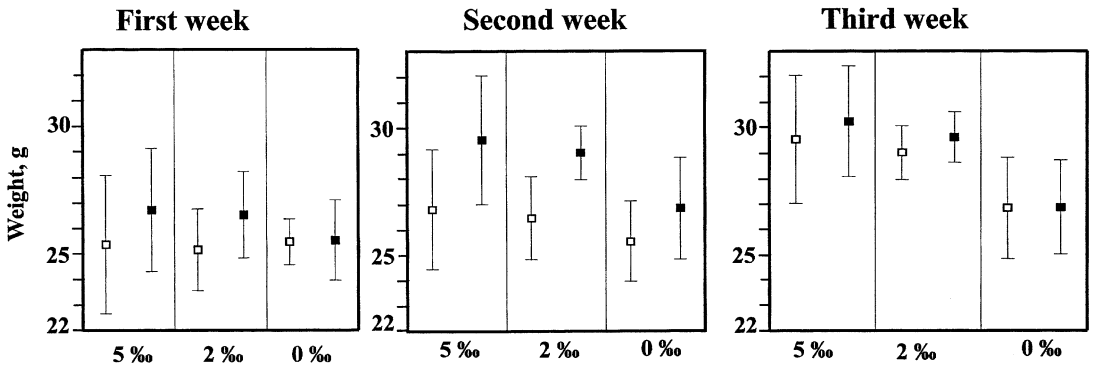

Figure 5. Weight increments of perch YOY during the first, second and third week (□ – beginning, ■ – end of the week) in brackish and freshwater (5 psu vs. freshwater, $p < 0.01$; 2 psu vs. freshwater, $p < 0.01$; 5 psu vs. 2 psu, $p > 0.05$).

as an increase in length, then growth is not possible below 10 °C (Karås, 1990). Consequently, good condition of fish may be assumed to result in growth in length later. Hence, both body size and condition characteristics can be used as indicators of growth.

Water temperature and food availability are generally considered to be the most important factors influencing growth rate in fish. However, salinity may play an important role as well, directly or indirectly influencing the growth of the studied species in the Curonian Lagoon and the Baltic Sea. High temperatures may stimulate growth directly by accelerating metabolism or indirectly by increasing food availability (Karås & Neuman, 1981). Le Cren (1958) demonstrated that the direct influence of temperature on perch growth is more important than the indirect influence via the food. Perch also exhibited a poorer development at low temperatures despite optimal availability of food (Kudrinskaja, 1970) and a correlation between length increment and temperature in the field has been demonstrated (Karås & Neuman, 1981). Large differences in growth rates between pikeperch populations living in lakes in northern and southern Europe have been reported (Deelder & Willemsen, 1964; Sonesten, 1991, cited in Lehtonen et al., 1996) and it was also shown experimentally that the growth rate of adult pikeperch in a heated pond was faster (Wilkońska, 1988). The positive influence of temperature on the growth of percids in the current study supports these previous observations. The growth of perch and pikeperch in the lagoon and the sea is slower during cold summers and faster during warm summers (Ložys, unpubl. data). However, the comparison of body size and condition characteristics in percid fishes between the Curonian Lagoon and the coastal waters of the Baltic Sea examined during the same seasons revealed that growth in brackish waters can be better under lower water temperatures. For the duration of the growing season (March–August) the water temperatures in the Curonian Lagoon were on average 2.4 °C higher than that in the sea. It is noteworthy that the water temperatures in the Curonian Lagoon during this study never exceeded the range of optimal temperatures for perch and pikeperch (Hokanson, 1977; Willemsen, 1977; Hilge, 1990; Karås & Thoresson, 1992). Abundant migrations have been observed in the Klaipeda strait towards the sea in spring and back to the lagoon in the autumn. Therefore it was assumed that the majority of fish caught from coastal zone spent the growing season in the Baltic.

In order to make a comparison of growth between the studied sites, a comparison of food availability is necessary. Available information suggests that feeding conditions in the Curonian Lagoon may be better than those in the coastal waters. The abundance of fish juveniles in the lagoon is significantly higher (mean g per 100 $m^2 \pm$ SD: 4025 $\pm$ 572, dominant species: perch, roach, gudgeon, vs. 502 $\pm$ 431, dominant species: smelt, sprat, and sand eel, $t = 11.8$, $p < 0.0001$; Repečka et al., 1996). Test fishing with 17-mm and 22-mm mesh size gill nets was conducted at both sites in 2000 and 2001. Significantly higher CPUE (30 m of net per 8 h) was established in the Curonian Lagoon (CPUE: 5707 $\pm$ 2184 vs. 1123 $\pm$ 596, in g, $t = 7.01$, $p < 0.0001$; Ložys, unpubl. data). However, pikeperch and perch of the studied age not only consume fish, but also different species of benthic invertebrates (Bubinas & Ložys, 2000). A zoobenthos study in the lagoon and the Lithuanian coastal zone down to the depth 40 m (Olenin, 1997), demonstrated that invertebrate biomass (molluscs excluded) was greater in the lagoon (Curonian Lagoon: 11.8 g m^{-2}; Baltic Sea:

5.5 g m^{-2}). Hence, the available information suggests that slower growth of percid fishes in the Curonian Lagoon would not be the result of lower food availability. Unfortunately, the energy content of prey (which also could influence the growth rate of fish) from the two sites has not been measured. Alternatively, the highly eutrophic nature of the Curonian Lagoon may negatively influence the consumption rate of a visual predator such as perch, while pikeperch are favoured in such systems since they have visual adaptations enhancing their foraging capacity in turbid environments (Karås & Sandström, 2002). A further possibility is that the fish in best condition are more likely to migrate from the lagoon to the sea and the sampling regime only sampled those fish remaining after such migrations.

The experimental results on the salinity effect on percid growth supported the hypothesis that brackish water positively influences perch YOY growth when temperature and food are controlled. This effect has also been demonstrated in some other studies, on larvae and juveniles of rainbow trout (*Salmo gairdneri*) (Tsintsadze, 1991), carp (*Cyprinus carpio*) (Tchizhik & Ryzhnikov, 1973; Tiutiunik, 1961) and larvae and juveniles of carp and silver carp (*Ctenopharyngodon idells*) (Konstantinov & Martynova, 1990, 2000). A positive effect of brackish waters on growth has also been demonstrated for typical marine fish species (Lambert et al., 1994; Gaumet et al., 1995; Woo & Kelly, 1995; Foss et al., 2001; Imsland et al., 2001). The beneficial effect of salinity on fish growth is most likely manifested only under salinities close to the internal osmotic pressure in fish. Some studies suggest that an isosmotic environment could be beneficial for both freshwater and marine fishes (Pic, 1978; Hales et al., 1990; Mazik et al., 1991). According to Brett (1979) and Jobling (1994, cited in Foss et al., 2001), marine fish species must spend energy to meet the metabolic cost of ionic and osmotic regulation. Consequently, it has been suggested that growth and food conversion may be improved in an isosmotic environment (Foss et al., 2001). Bein & Ribi (1994) suggested that since freshwater fish are hypertonic with respect to freshwater, a low concentration of salt in the surrounding might facilitate the osmoregulation in larval fish, which have a large surface to volume ratio. Contrarily, the standard metabolic rate determined at a salinity of 5 psu was close to that in freshwater (Solomon & Brafield, 1972; Karås, 1996). Others did not find an impact of salinity below 6 psu on the metabolism of perch fry (Toneys & Colbe, 1980). Accordingly Karås (1996) suggested that at typical salinities of 4 and 7 psu (prevalent Baltic coastal waters) probably could be ruled out as modifying the effect of temperature on metabolism, growth and survival.

The results of the current study provides evidence suggesting that low salinities can beneficially affect the growth of percid fish. Furthermore, the obtained results indicate that under certain circumstances (equal experimental temperature and feeding conditions), an effect of salinity can be seen. Salinity has been relatively neglected in percid energetic studies during the last decades. The obtained results suggest that salinity is a significant abiotic factor and should be taken into account in percids fish biology studies in the brackish environments. The mechanisms of the impacts of brackish water on freshwater fish are still unclear and require further investigation.

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Effects of temperature on egg and larval survival of cod (*Gadus morhua*) and sprat (*Sprattus sprattus*) in the Baltic Sea – implications for stock development

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Key words: cod, sprat, temperature, vertical egg distribution, egg survival, larval viability, Baltic Sea

Abstract

Stock development of cod and sprat, two major fish species in the Baltic Sea, is linked by trophic interactions. Depending on recruitment success the Baltic may be pushed towards either a cod- or a clupeid dominated system. Both cod and sprat spawn in the Baltic deep basins at strongly varying hydrographical conditions with survival during the egg and early larval stages regarded as a major bottleneck. Due to differences in egg specific gravity, cod and sprat eggs occur at different depths and are thus subject to different hydrographical conditions. For sprat, weak year-classes have been associated with low water temperatures during peak spawning. For cod the shift in peak spawning from spring to summer during the 1990s has been discussed as a reason for the poor recruitment at present as delayed spawning may involve egg development at too high temperatures. In the present study cod and sprat eggs and yolk sac larvae were incubated at different temperatures, 1–11 °C for cod and 1–13 °C for sprat. No difference in viable hatch occurred in the range 3–9 °C for cod and in the range 5–13 °C for sprat. Larval viability decreased at 11 °C for cod and at $\leq$5 °C for sprat. Comparing the results with vertical egg distribution and temperature profiles from field studies suggested no major influence of temperature on cod reproduction, but a considerable effect on sprat. The results imply that different environmental conditions; frequency of major saline water inflows into the Baltic Sea for cod, and water temperature in the upper layers, e.g. following severe/mild winters, for sprat, involve different opportunities for egg and larval survival and may thus cause a displacement in the balance between cod and sprat.

Introduction

Cod, sprat and herring are the major fish species in the Baltic Sea both in the fishery as well as from an ecological point of view, cod being the dominant piscivore species and sprat and herring the major planktivorous fish species (e.g. Arrhenius & Hansson, 1993; Thurow, 1993; Sparholt, 1994). The stocks are linked by trophic interactions; cod prey on sprat and herring, and mainly sprat but also herring prey on cod eggs and early larvae (Sparholt, 1994; Köster & Möllmann, 2000a). As the distributions of cod and sprat largely overlap, stock development of cod and sprat is closely related (e.g. Sparholt, 1996). During the 1980s cod were abundant whereas sprat and herring dominate the Baltic ecosystem at present. Thus, the Baltic fish community can be shifted to a system dominated by either cod or clupeids, potentially affecting lower trophic levels by top down control (Rudstam et al., 1994; Köster & Möllmann, 2000a). The fishery may push the system towards either a cod or a clupeid dominated system [e.g. a fishing mortality on cod of 0.9–1.4 during the period 1986–1992 (ICES, 1999)]. Potentially environmental variability may cause such a shift as hydrographical conditions in the Baltic deep basins, where a majority of marine fish species including cod and sprat reproduce, vary greatly depending on frequency and magnitude of saline water inflows (Grauman & Yula, 1989; Bagge & Thurow, 1994; Paramanne et al., 1994; MacKenzie et al., 2000). Due

to differences in egg specific gravity, cod and sprat eggs occur at different depths; cod eggs in and below the halocline whereas sprat eggs are distributed also in the upper water (e.g. Grauman, 1965; Nissling et al., 1994) indicating that eggs and early larvae of cod and sprat are subject to different hydrographical regimes.

Temperature affects egg and larval performances, e.g. developmental rate, growth and mortality, but also food availability due to variability in zooplankton production (the match-mismatch hypothesis, e.g. Cushing, 1982). Poor year classes of Baltic sprat have been associated to severe winters accompanied with low water temperatures during peak spawning (Paramanne et al., 1994) and concern about negative effects of high water temperatures on egg and larval development for Baltic cod has been discussed since the shift in peak spawning from spring to summer during the 1990s (e.g. Wieland et al., 2000). Thus, to define (sprat) and re-define (cod) the reproductive volume (see Plikshs et al., 1993), information about viability of eggs and larvae in relation to temperature is required. Up to now no such data for the Baltic populations exist. In the present study, eggs and yolk sac larvae of Baltic cod and sprat were incubated experimentally at different temperatures, and egg distribution in relation to temperature regimes was examined by combining data of egg specific gravity for respective species and hydrographical data from CTD runs in the main spawning areas.

Material and methods

Cod

Cod were caught by trawling in SD 27 and SD 28, transported to the Ar laboratory, Gotland, Sweden and kept in indoor plastic pens (12 m^3) provided with running seawater (6.5–7 psu; 4–11 °C). Eggs and semen were obtained by stripping and fertilisation was carried out at 7 °C and 17 psu (positive egg buoyancy) prepared from filtered (0.2 μm cartridge filter) seawater and synthetic sea salt as described in Nissling et al. (1998). Only early batches (batch 1–5) were used in experiments to ensure high quality eggs (e.g. Solemdal et al., 1995). At stage IA (Thompson & Riley, 1981), a sub-sample of eggs was examined under a stereomicroscope. Eggs with regular or irregular development (Kjørsvik et al., 1990), were sampled and incubated in duplicate, using 150 eggs in each, in 300 ml 17 psu water treated with antibiotics (Doctacillin 0.1 g l^{-1}; Streptomycin 0.05 g l^{-1}; Nystatin 2500 IU l^{-1}). 2/3 of the water was exchanged daily and dead eggs were counted and removed. In total 9 egg batches from different females were incubated in water baths at five temperatures regulated using thermostats (Julabo P/EM; temperature stability ± 0.03 °C). The average temperatures during incubations were 1.0, 2.9, 7.3, 9.0 and 10.8 °C, respectively. At the onset of hatching the incubations were placed in darkness until ≥ 50% hatching (at on average day 33–35, 24–25, 14, 12 and 10 at 1, 3, 7, 9 and 11 °C respectively). When hatching was completed (all eggs died or hatched), the viable hatch, i.e. the number of larvae with normal swimming functions, was assessed. Hatched larvae were incubated as described above in duplicate, using 50 larvae in each, from the time of 50% hatching until yolk absorption; at day 6–8 at 11 °C; day 7–9 at 9 °C; day 8–10 at 7 °C; day 15–18 at 3 °C and at day 22–26 at 1 °C. At the same occasions, larvae were preserved in neutral 4% formaldehyde and size [standard length (Fossum, 1988)] was measured under a stereomicroscope at ×20 magnification. To evaluate opportunities for feeding, the time from functional jaw to yolk sac absorption was assessed.

Sprat

Eggs were obtained from SD 26 and SD 28 in two ways, either from stripping fish caught by pelagic trawling, or from *in situ* sampling using a 'living net' (modified WP-2) towed vertically through the water column. Eggs and semen were sampled on fish directly after catch or the following night on fish being kept in tanks provided with running seawater (≈ 7 psu) and fertilisation was carried out by mixing eggs and semen at 15 psu (positive egg buoyancy) at 7 °C. About 1–2 h after fertilisation, eggs were transferred to new water at the same conditions. The eggs were transported to the Ar laboratory, Gotland, Sweden and incubated from stage IA–IB at 15 psu using the same method as described for cod.

Two incubation series were performed. In the first series, egg and larval performances were assessed at 7 different temperatures, at on average 0.9, 3.2, 5.2, 7.0, 8.8, 10.8, 12.9 °C. Four batches of 80–100 eggs, obtained from either stripping ($n = 2$) or from living net sampling ($n = 2$), were incubated at each temperature. Hatched larvae were judged as viable or nonviable using the same criteria as for cod and summed until hatching was completed (at on average day 16, 11, 8, 7, 6 and 5 at 3, 5, 7, 9, 11 and 13 °C respectively).

At approximately 50% hatching 10–20 hatched larvae were preserved in neutral 4% formaldehyde for size measurements. The remaining larvae were incubated further at the same conditions as described above until completion of the yolk sac (at on average day 17, 13, 10, 8.5 and 6.5 at 5, 7, 9, 11 and 13 °C, respectively). The time from functional jaw to yolk sac absorption was assessed, and the remaining larvae were preserved for size measurements. These were performed under a stereomicroscope at ×30 (at hatching) and ×15 (at completed yolk sac) magnification using a micrometer scale. In the second series, eggs obtained from living net sampling in SD 28 were incubated from stage IA-IB in 5 replicates at 4 temperatures at on average 2.0, 3.1, 4.0, 5.1 °C as described above, and the viable hatch was assessed.

Egg specific gravity

Information about egg specific gravity was taken from Nissling & Westin (1997) (cod) and Nissling et al. (2003) (sprat). Egg specific gravity was determined by using a density gradient column for eggs in the blastula stage obtained by stripping and artificial fertilisation from 71 and 50 different cod and sprat females, respectively, caught in SDs 25, 26 and 28.

Egg specific gravity was tested for normal distribution using a Goodness of fit (Sokhal & Rohlf, 1981) [$x^2 = 10.06$, $df = 5$, $0.1 > x > 0.5$ (cod); $x^2 = 2.86$, $df = 2$, $0.3 > x > 0.2$ (sprat caught during peak spawning in May/June)] and the share of eggs with specific gravity at a certain level was calculated using average ± standard deviation [1.01175 ± 0.00109 g cm^{-3} (cod); 1.01091 ± 0.00078 g cm^{-3} (sprat in April); 1.00858 ± 0.00116 g cm^{-3} (sprat in May/June)] and z-distribution. As Bartlett's test for homogeneity of variances (Dixon & Massey, 1983) showed equal variance in egg specific gravity for respective species/sampling occasion, the standard deviation for cod ($n = 71$) was used in all cases.

Hydrographic conditions

Information about water density and temperature conditions in the spawning areas in the year 2000 was obtained from IFM Kiel, Germany (EU STORE-project FAIR CT98 3959), assessed by CTD runs carried out at the same occasions as the sprat were sampled for stripping and egg specific gravity measurements were performed.

Results

Egg and larval viability

Cod: Baltic cod egg and larval performances at different temperatures are shown in Table 1. As large differences in viable hatch among females occurred, coefficient of variation ∼47%, potential differences between temperatures were analysed within females (R × C tables using G-test). No difference in viable hatch occurred at 3–9 °C (Table 2) whereas significantly lower viable hatch was recorded at both 1 °C and 11 °C in 7 out of 9 incubations. Larval survival during the yolk sac stage was significantly lower at 11 °C ($df = 4$, $x^2 = 11.0$, $p < 0.05$) compared to at 1–9 °C ($df = 3$, $x^2 = 4.83$, $0.10 < p < 0.05$). By applying a procedure of hatching in darkness (Nissling et al., 1998), most larval groups were sampled at approximately the same stage of development. Despite this, examination of developmental stage under a stereomicroscope revealed that sampling for some larval groups was not carried out at the same stage. Those larval groups were rejected when analysing potential differences in larval size. An ANOVA with 'structural zeros' (STATISTICA) was applied to evaluate differences in larval length (Table 1). At hatching, larval size was significantly lower at 11 °C and significantly higher at 1 °C, $F_{1:504} = 32.6$, $p < 0.0001$ and $F_{1:504} = 89.0$, $p < 0.0001$, respectively, whereas no differences occurred in the range 3–9 °C ($F_{2:299} = 1.30$, $p = 0.27$). Larval size at consumed yolk sac differed significantly among temperatures, $F_{4:529} = 78.7$, $p < 0.0001$. The larvae were somewhat larger at low, 1 °C and 3 °C, temperatures ($F_{1:301} = 16.7$, $p < 0.0001$; 7–9 against 1 °C and $F_{1:349} = 69.3$, $p < 0.0001$; 7–9 against 3 °C; Sheffe's contrast test; STATVIEW), and significantly smaller at high, 11 °C, temperatures ($F_{1:532} = 220$, $p < 0.0001$; 1–9 against 11 °C). No differences were recorded between 7 °C and 9 °C ($F_{1:202} = 0.22$, $p = 0.640$). This suggests that egg survival is affected negatively at both low (1 °C) and high (11 °C) temperatures and that larval performances are unaffected in the range 1–9 °C, but significantly worse at 11 °C. The time from functional jaw to yolk sac absorption varied with temperature; on average ∼3, 3.5, 4, 8.5 and 13 days at 11, 9, 7, 3 and 1 °C, respectively. Thus, the time from start of feeding until exhausted yolk was considerably shorter at high temperatures than at lower temperatures, implying a higher demand for food availability at high temperatures.

Table 1. Viable hatch and larval survival during the yolk sac stage (also shown in relation to survival at 7 °C), and larval length (average of female means) at hatching and after yolk sac consumption of Baltic cod (*Gadus morhua*) at different temperatures. Average of 9 set-ups with eggs from different females (± standard deviation). Larval length was measured after preservation in 4% neutral formaldehyde.

Temperature (°C)	Viable hatch hatch (%)	Relative viable hatch (%)	Larval length at hatching (mm)	Larval length at yolk sac consumption (mm)	Larval survival (%)	Relative larval survival (%)
1	37.7 ± 21.7	71.4 ± 41.1	3.82 ± 0.13	4.07 ± 0.17	85.7 ± 9.1	95.2 ± 10.1
3	52.4 ± 22.5	99.2 ± 42.6	3.72 ± 0.15	4.12 ± 0.13	85.3 ± 10.1	94.8 ± 11.2
7	52.8 ± 21.7	100 ± 41.0	3.72 ± 0.11	4.02 ± 0.13	90.0 ± 8.0	100 ± 8.9
9	50.8 ± 19.0	96.2 ± 36.0	3.73 ± 0.11	4.02 ± 0.12	87.8 ± 7.9	97.6 ± 8.8
11	39.9 ± 22.7	75.6 ± 43.0	3.57 ± 0.12	3.83 ± 0.13	75.7 ± 19.1	84.1 ± 21.2

Table 2. Outcome of statistical analyses (R×C tables using G-test) of viable hatch of Baltic cod (*Gadus morhua*) at different temperatures. 9 set-ups with eggs from different females were used.

Female	Significant		Not significant	
	°C	*p*-value	°C	*p*-value
1	1, 3, 7, 9, 11	<0.0001	3, 7, 9	0.209
2	1, 3, 7, 9, 11	<0.0001	3, 7, 9	0.065
3	1, 3, 7, 9, 11	0.0006	3, 7, 9, 11	0.100
4	1, 3, 7, 9, 11	<0.0001	3, 7, 9	0.079
5	1, 3, 7, 9, 11	<0.0001	1, 3, 7, 9	0.094
6	1, 3, 7, 9, 11	<0.0001	3, 7, 9	0.896
7	–	–	1, 3, 7, 9, 11	0.082
8	1, 3, 7, 9, 11	<0.0001	3, 9	0.179
9	3, 7, 9, 11	<0.0001	3, 7, 9	0.225

Sprat: In Table 3, viable hatch and larval performances of Baltic sprat at different temperatures are shown. No differences in viable hatch was recorded at 5–13 °C ($df = 4$, $G = 8.1$, $p = 0.087$), whereas significantly lower viable hatch occurred at 1 °C and 3 °C ($df = 6$, $G = 721.9$, $p < 0.001$ and $df = 5$, $G = 204.2$, $p < 0.001$, respectively). Larval length at hatching differed between temperatures, 3–13 °C ($F_{5:138} = 10.0$, $p < 0.001$; ANOVA), with significantly lower larval size at 3 °C (Sheffe's contrast test; STATVIEW). Larval development was studied for the two groups obtained from stripped eggs (assessment of larval development at 1 °C and 3 °C was not possible due to only a few hatched larvae). The results strongly indicate decreased opportunities for larval survival of Baltic sprat at low temperatures. Larval survival was significantly lower at 5 °C compared to 7–13 °C ($df = 4$, $G = 30.5$, $p < 0.001$ and $df = 3$, $G = 7.21$, $p = 0.065$ respectively), as was larval size at yolk sac completion ($F_{4:98} = 3.52$, $p = 0.010$; with lower size at 5 °C compared to 7–13 °C, Sheffe's contrast test; STATVIEW). Furthermore, the time from functional jaw to completed yolk sac differed only slightly, from ∼1 to ∼3 days at 13 °C and 5 °C, respectively, indicating the importance of a match between the time of functional jaw and food availability.

As sprat eggs in the Baltic regularly occur at temperatures of 3–4 °C (see below), a second incubation series was performed at temperatures ≤5 °C (Table 4; incubation series 1 and 2 pooled). Viable hatch decreased significantly with temperature, with a considerably decrease between 4 °C and 3 °C (less than 50% at 3 °C compared to 4 °C), suggesting that water temperatures of ≤4 °C may negatively affect the reproductive success seriously.

Vertical distribution

Cod: Vertical egg distribution and temperature profiles in the Bornholm basin, the main spawning area for Baltic cod (Bagge et al., 1994), at three occasions during the spawning season are shown in Fig. 1. Eggs were distributed from 55–60 m depth to the bottom at 90 m at all occasions, with peak egg abundance at ∼6.5–7 °C. Despite an increase in temperature in the surface layer during the season, cod eggs were not subject to temperatures >9 °C. Rather, eggs were exposed to increased temperatures, ∼9 °C, in the near-bottom water in April 2000 (Fig. 1a). This suggests that cod eggs are not likely to be affected by high water

Table 3. Viable hatch, larval survival during the yolk sac stage and larval length (measured after preservation in 4% neutral formaldehyde) at hatching and after yolk sac consumption of Baltic sprat (*Sprattus sprattus*) at different temperatures (± standard deviation).

Temperature (°C)	Viable hatch[a] (%)	Coefficient of variation (%)	Larval length at hatching[a] (mm)	Larval length at yolk sac consumption[b] (mm)	Larval survival[b] (%)
1	0.2 ± 0.5	250	–	–	–
3	24.2 ± 7.9	32.6	2.43 ± 0.09	–	–
5	60.6 ± 4.9	8.1	2.56 ± 0.11	4.30 ± 0.26	61.4 ± 22.5
7	65.1 ± 14.1	21.6	2.55 ± 0.18	4.48[c]	97.8 ± 3.2
9	68.6 ± 10.3	15.0	2.55 ± 0.13	4.44 ± 0.28	91.7 ± 11.8
11	68.1 ± 3.2	4.7	2.65 ± 0.16	4.54 ± 0.33	86.7 ± 18.9
13	62.1 ± 4.7	7.6	2.63 ± 0.08	4.58 ± 0.28	96.9 ± 4.4

[a] $n = 4$ (2 with eggs obtained by stripping and 2 with eggs from *in situ* sampling).
[b] $n = 2$ (eggs obtained by stripping).
[c] $n = 1$.

Table 4. Viable hatch (also shown in relation to survival at 5 °C), coefficient of variation and significance level (R×C tables using G-test) of Baltic sprat (*Sprattus sprattus*) at different temperatures (± standard deviation).

Temperature (°C)	Viable hatch (%)	Relative viable hatch (%)	Coefficient of variation (%)	G (df)	Significance level
2[a]	7.9±8.8	11.6±12.9	111	327[a]	$P < 0.001$
3[b]	26.0±11.8	38.3±17.4	45.4	182[b]	$P < 0.001$
4[a]	55.9±4.2	82.3±6.2	7.5	13.0[a]	$P < 0.01$
5[b]	67.9±5.8	100±8.5	8.5		

[a] $n = 5$ (eggs obtained from *in situ* sampling).
[b] $n = 9$ (2 with eggs obtained by stripping and 7 with eggs from *in situ* sampling).

temperatures occurring in the upper layers at delayed spawning.

Sprat: Vertical egg distribution and temperature regimes in the Bornholm basin in April, and in the Bornholm basin, the Gdansk deep and the Gotland basin during peak spawning in May/June are shown in Fig. 2. As there is a change in egg specific gravity during the season, there is accordingly a shift in vertical egg distribution, e.g. from 50–80 m in April to 25–65 m in May/June in the Bornholm basin (Fig. 2a, b). Accordingly, the risk of being exposed to low temperatures also changes. In the Bornholm basin, where the eggs were distributed in the deep layers early in the season, the most buoyant eggs occurred at temperatures <4 °C and almost 50% of the eggs were subjected to temperatures <5 °C (Fig. 2a). During peak spawning (Fig. 2b), no eggs in the Bornholm basin occurred at temperatures <4 °C but the majority (≥75%) was found at depths with a temperature below 5 °C. In the Gdansk deep and the Gotland basin (Figs. 2c, d), approximately 20% and 10% of the eggs, respectively, occurred at temperatures below 4 °C, and ∼40% and ∼50%, respectively, at depths with temperatures <5 °C at the time of peak spawning. This suggests that sprat eggs in the Baltic regularly occur at depths where temperature may affect egg and early larval viability.

Discussion

Egg survival of Baltic cod was unaffected in the range 3–9 °C but significantly lower at 11 °C. This is in accordance with other investigations, e.g. a decrease in egg survival at 12 °C but not at 10 °C has been reported for Norwegian coastal cod (Iversen & Danielssen, 1984), and a high percentage of viable hatch was found in the range 2–10 °C compared to 12 °C for cod from the Gulf of Main area (Laurence

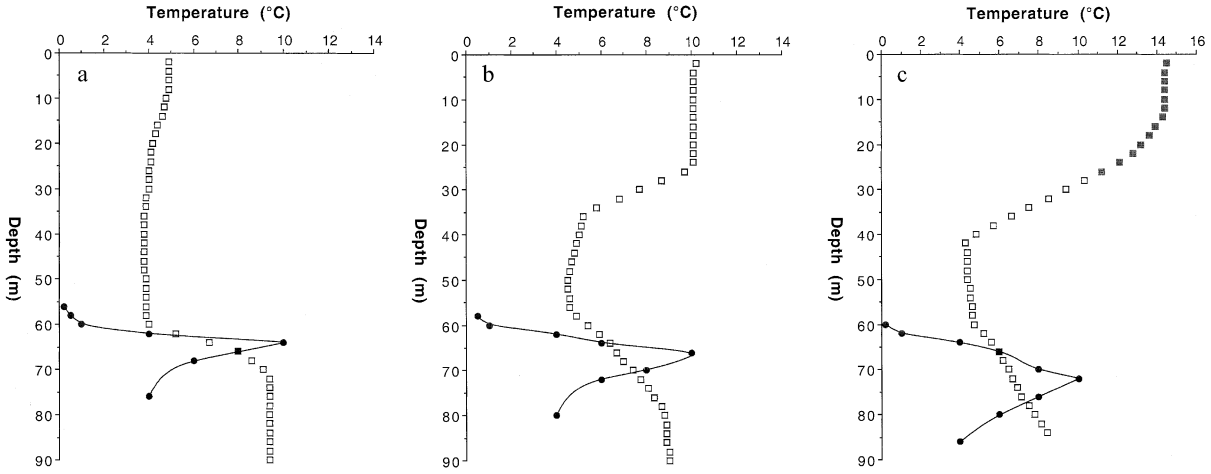

Figure 1. Vertical distribution of Baltic cod (*Gadus morhua*) eggs and temperature profiles in the main spawning area, the Bornholm basin, in April (a), May/June (b) and July (c) in the year 2000 derived from CTD runs and egg specific gravity measurements of stripped eggs (Nissling & Westin, 1997) with applied *z*-distribution. Dark squares referring to unfavourable temperatures.

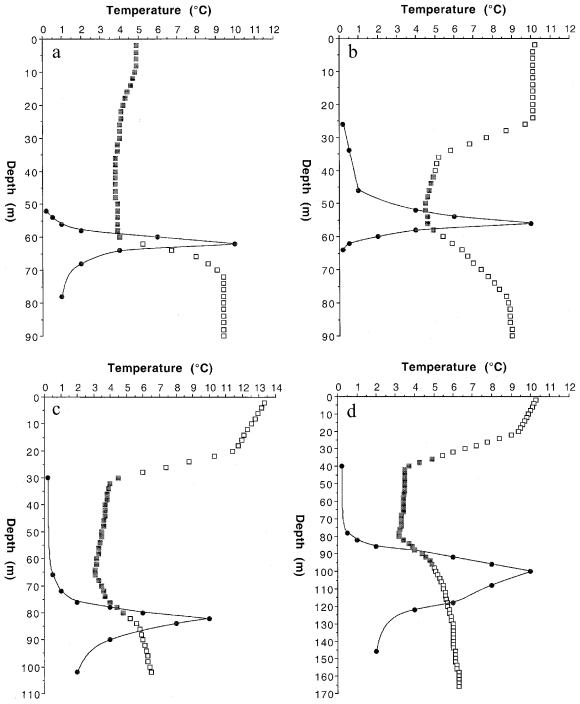

Figure 2. Vertical distribution of Baltic sprat (*Sprattus sprattus*) eggs and temperature profiles in the main spawning areas in the year 2000, the Bornholm basin in April (a), the Bornholm basin in May/June (b), the Gdansk deep in May/June (c) and the Gotland basin in May/June (d) derived from CTD runs and egg specific gravity measurements of stripped eggs (Nissling et al., 2003)) with applied *z*-distribution. Dark squares referring to unfavourable temperatures.

& Rogers, 1976). That differences in the ability to withstand low temperatures exist among cod populations is obvious, e.g. eggs of cod off Newfoundland may develop successfully below zero due to antifreeze proteins (Valerio et al., 1992) whereas Thompson & Riley (1981) reported no egg development at temperatures below 1.5 °C for North Sea cod. In the present investigation, Baltic cod eggs were shown to survive at 1 °C, despite a lower viable hatch.

The present investigation revealed lower viable hatch of Baltic sprat at 1–4 °C but no difference in the range 5–13 °C. This is somewhat different to the temperatures reported by Thompson et al. (1981) for North Sea sprat. They found considerably lower survival to hatching at 4 °C and 5 °C compared to at 6–18.5 °C. In the present investigation, sprat eggs were incubated up to 13 °C without any effect on survival. As sprat eggs in the Baltic Sea, due to failure in obtaining neutral egg buoyancy at temperatures above ~13–14 °C in brackish-water conditions of 7–8 psu (Nissling et al., 2003), effects of higher temperatures should not be expected.

Temperature affects both size and energy reserves at hatching, as well as developmental rate, yolk consumption and growth rate (e.g. Iversen & Danielssen, 1984; Kuftina & Novikov, 1986; present investigation) influencing larval survival probabilities. Temperature also affects larval survival indirectly in terms of food availability (see Ellertsen et al., 1989; Cushing, 1990). The present investigation shows lower larval viability for cod during the yolk sac stage at high temperatures (11 °C) and significantly shorter duration of the devel-

opment from functional jaw to yolk sac absorption at high temperatures. For sprat, the present study suggests lower larval viability at temperatures of ≤5 °C and a short duration of the development from functional jaw to yolk sac absorption, only 3 to 1 days, at 5 to 13 °C.

Some differences in larval development occurred between cod and sprat. Cod showed an increase in larval size at low temperatures (1 °C and 3 °C), and a decrease in both size and viability at high (11 °C) temperatures. Contrarily, sprat displayed poorer larval growth and viability at low (≤5 °C) temperatures indicating a difference in optimal temperatures between the species. For cod, being a cold-water species (see Brander, 1994), development is hampered by increased energetic costs at high temperatures whereas sprat, being a warm water species (see Paramanne et al., 1994), is negatively affected at low temperatures. Furthermore, developmental time differed between the species. At e.g. 9 °C, the time until hatching was considerably longer for cod (∼12 days) compared to for sprat (∼7 days) whereas the time from hatching to yolk sac absorption was shorter for cod (∼8 days) than for sprat (∼10 days). However, considering the time of onset of feeding, i.e. when the jaw is functional, the time was approximately the same for both species, about day 15 after fertilisation. Differences occurred also in terms of growth. For cod with a comparatively long development to hatching, larval length increased by on average 8% until yolk sac completion, whereas for sprat length increased by more than 70%. Apparently, sprat allocate relatively more energy resources into growth in length resulting in a short time from functional jaw to yolk sac absorption suggesting that sprat larvae are highly vulnerable to a mismatch between time of hatching and occurrence of prey items (see Cushing, 1982, 1990). As larvae migrate to the upper layers at the onset of feeding (e.g. Grønkjær & Wieland, 1997; Rohlf, 1999), this indicates that cod larvae may be affected negatively late in the season whereas sprat larvae may face unfavourable temperature conditions early in the season (see Figs. 1c and 2a).

Vertical egg distribution of both cod and sprat in the Baltic Sea is not governed by water density and egg specific gravity only; it is also affected by mortality due to low oxygen concentrations in the deep basins (e.g. Nissling et al., 1994, 2003; Wieland 1995), by sub-optimal temperatures (present study) and by predation (Köster & Möllmann, 2000a,b). In contrast to *in situ* sampling, the approach used in present investigation (specific gravity of eggs obtained by stripping and applied z-distribution) reflects the initial vertical egg distribution, i.e. it shows the conditions at which egg development will occur, enabling an evaluation of the share of eggs subjected to unfavourable conditions.

In accordance with differences in egg specific gravity, the present investigation suggests no effect of temperature on the viable hatch for cod in the Baltic Sea, but that egg survival of sprat may be highly influenced by low temperatures. Cod eggs keep buoyant in and below the halocline at more or less stable temperature conditions whereas eggs of sprat inhabit layers with highly varying temperatures, both between years and during the spawning season. In ichtyoplankton sampling in the Bornholm basin, cod eggs were found at temperatures ranging from 0.4–7.1 °C (on average 2.7–6.4 °C) during the period 1986–1996 (Wieland & Jarre-Teichmann, 1997). At no occasion, cod eggs were found at temperatures >9 °C. MacKenzie et al. (1996) estimated average water temperatures experienced by cod eggs in the Bornholm basin to 5.5–6 °C, irrespective of time of spawning. Concerning sprat, ichtyoplankton sampling has shown that eggs may occur in a wide range of temperatures in accordance with the change in egg specific gravity during the spawning season. In the Bornholm basin, peak egg abundance occurred from ∼1.5 °C to 6 °C during 1987–1990 (derived from figures in Wieland & Zuzarte, 1991) with mean weighted relative abundance ranging from 3.6 °C to 6.8 °C (Wieland & Zuzarte, 1991). Clearly, sprat eggs regularly occur at depths with unfavourable water temperatures, influencing egg and larval viability. The above suggest that temperature should be considered in the definition of the reproductive volume for sprat, whereas effects on reproduction of cod are limited. Thus, inclusion of temperature as a parameter in defining the reproductive volume for cod would probably not increase recruitment relationships (see Jarre-Teichmann et al., 2000).

The reproduction of both cod and sprat may be affected by poor oxygen conditions in the deep basins, which varies between years and spawning areas. For cod an oxygen level of ≥2 ml l^{-1} is required (Nissling, 1994; Wieland et al., 1994). However, due to higher egg specific gravity, cod eggs are more regularly exposed to low oxygen conditions than are sprat eggs. Renewal of oxygen stores in the deep layers occur only following 'major' inflow events of saline water whereas layers inhabited by sprat eggs are more regularly ventilated as most interleaving occur in the halocline region (Stigebrandt, 2001).

Cod and sprat are two major fish species in the Baltic Sea, and a displacement in the balance of these species may affect not only the fishing community, but also the Baltic ecosystem. Results from the present investigation, as well as previous work concerning cod reproduction (Plikshs et al., 1993; Nissling et al., 1994; MacKenzie et al., 2000), suggest that different environmental conditions, magnitude and frequency as well as quality (salinity conditions) of inflow events into the Baltic, influencing salinity and oxygen conditions in the deep layers (for cod), and water temperature in the upper layers, e.g. following severe or mild winters, respectively (for sprat), involve different opportunities for egg and larval viability. The 'great stagnation period' without major saline water inflows between 1985–1993 (Franck & Matthäus, 1992; Matthäus, 1993; Matthäus & Lass, 1995) resulted in poor conditions (e.g. MacKenzie et al., 2000) and weak year classes of cod (Bagge et al., 1994), whereas a series of years with temperatures above average during the 1990s (Alexandersson, 2001) yielded strong year classes of spat (ICES, 1999). This suggests that large-scale climate variations (see Lehmann et al., 2001) may push the Baltic towards a cod or a clupeid dominated system.

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Influence of dissolved organic matter from terrestrial origin on the changes of dinoflagellate species composition in the Gulf of Riga, Baltic Sea

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Key words: DOM, dinoflagellates, *Dinophysis acuminata*, *Protoperidinium brevipes*, growth rate

Abstract

A mesocosm experiment was used to investigate the effect of terrestrial-origin dissolved organic matter (DOM) on the development of dinoflagellates in natural summer phytoplankton from the Gulf of Riga. Seawater was collected in the central part of the Gulf of Riga and at the entrance of the Gulf in June 1999. DOM was extracted from Pärnu River water by use of tangential ultrafiltration. Experimental series were enriched with DOM, DOM in combination with nitrate and phosphate, and only with inorganic nutrients. Enrichments were added in ranges of their natural concentrations. *Dinophysis acuminata, Protoperidinium brevipes* and *Gymnodinium* spp. were dominant species in the initial dinoflagellate community. During the experiment, the best growth of dinoflagellates was observed in treatments with DOM and DOM in combination with phosphate, mainly due to active growth of *D. acuminata* (maximum $\mu = 0.8$ day^{-1}). Significant uptake of dissolved organic nitrogenous compounds was seen, indicating the importance of heterotrophic nutrition among the phytoplankton species.

Introduction

The Gulf of Riga is a relatively enclosed water basin with the main freshwater inflows in its southern part and weak water exchange with the Baltic Sea. Between the 1980s and 1990s, the Gulf of Riga received annually on average 80.3 kT yr^{-1} of nitrogen and 2.3 kT yr^{-1} of phosphorus. The proportion of organic nitrogen in the loads was ~50% at the beginning of the year and reached almost 70% in June–August. Similar to phosphorus loads, the proportion of organic phosphorus increased between the period from January to July from 20% to 53%, and in autumn it decreased again to the January level (Andrushaitis et al., 1995). Since the 1990s, nitrogen concentrations have decreased while phosphorus concentrations have increased and phosphorus has lost its role as a limiting nutrient in the Gulf of Riga. At the same time, silicon deficiency has been observed during spring blooms (Rahm et al., 1996). Changes in the limiting nutrients have caused also significant changes in phytoplankton community structure. Due to reduced availability of nitrogen and probably also to increasing silicon limitation (Yurkovskis et al., 1999), a decrease of diatom abundance has been observed in spring, and well-expressed blooms of toxic cyanobacteria now occur in the summer period (Balode, 1994; Balode & Purina, 1996). In the Gulf of Riga, dinoflagellates are the major component of the phytoplankton assemblage in the transitional stage between spring and summer blooms (end of May–June), and often they accompany toxic cyanobacterial blooms in July- August. Maximal cell densities of dinoflagellates were observed to occur when the concentrations of inorganic nitrogen in the water column were close to analytical zero (Balode, 1994; Balode et al., 1998), but concentrations of DON

were up to 20–30 μM l^{-1} (Berg et al., 2001), suggesting that dinoflagellates profit from dissolved organic substances or organic particles.

The wide range of nutritional status of dinoflagellates is well known, ranging from pure autotrophs, mixotrophs to pure heterotrophs (Granéli & Carlsson, 1998; Hansen, 1991; Havskum & Riemann, 1996; Jacobson & Anderson, 1996). Gaines and Elbrächter (1987) reported that only six species are strictly autotrophic, while the other photosynthetic species are considered to be auxotrophic, mixotrophic in *sensu stricto* or even phagotrophic, but still others are non-photosynthetic. A continuous gradient between truly autotrophic and heterotrophic organisms probably exists (Granéli & Carlsson, 1998). Even species of the same genera can have different modes of nutrition (Larsen & Sournia, 1991). Some organisms initiate phagotrophy in the presence of sufficient quantities of prey, while for others phagotrophy might be induced by abiotic factors such as light or inorganic nutrients (Granéli et al., 1999).

Humic substances can affect coastal plankton communities by stimulating the growth rate and nitrogen yield of dinoflagellates (Carlsson et al., 1993, 1995, 1999; Granéli et al., 1999). This growth-stimulating effect has generally been attributed to the ability of humic substances to act as chelators, making trace elements available for phytoplankton (Prakash et al., 1973). Another hypothesis is that DOM is used by bacteria, increasing phytoplankton production via the 'microbial loop' (Azam et al., 1983). Experiments with *Alexandrium catenella* have shown that dinoflagellates are able to take up high-molecular-weight dextrans (2000 kDa) probably by pinocytosis, and addition of humic substances can enhance the uptake of dextrans (Legrand & Carlsson, 1998).

In our experiments, the dynamics of dinoflagellate populations were investigated in a mesocosm experiment using a natural phytoplankton community. The aim of the present study is to investigate the impact of DOM on the dinoflagellate communities of the Gulf of Riga and to compare the effects of N-DOM and N-NO_3 on the algal succession.

Materials and methods

A ship cruise was made on 11–12 June 2000 to collect seawater at two different stations, in the central part of the Gulf of Riga (St. 121) and in the open Baltic Sea at the entrance of the Gulf (St. 34a). Seawater for the experiments was collected in the surface layer of both stations.

Water for the DOM extraction was collected in Pärnu River, upstream from the town of Pärnu. River water was pre-filtered through 3 and 1 μm Millipore filter cartridges, then onto GF/C, GF/F (Whatmann), 0.45 μm nucleopore filters and 0.2 μm Opticap filter units (Millipore). DOM was concentrated by using a tangential ultra-filtration device, the Prep/scale^{TM} TFF 6 ft^2 cartridge (Millipore) (Guo et al., 1995; Benner et al., 1997). The fraction of DOM >1 kDa was used for experiments.

Seawater was pre-filtered on a 150-μm mesh to remove the large zooplankton, and filled in 19.6-l polycarbonate bottles (19 l per bottle). The experiment consisted of 5 treatments with triplicate bottles: (1) without nutrient addition (Control); (2) with addition of DOM (+DOM); (3) with addition of DOM and nitrates (+DOM+NO_3); (4) with addition of DOM and phosphate (+DOM+PO_4); (5) with addition of nitrate and phosphate (+NO_3+PO_4). The nutrient mixtures were added during a period of five days to obtain a final enrichment of 25 μM N and 1.6 μM P ('Redfield ratio'). No additions were made between days 6–10 (Table 1). Culture bottles were installed in outdoor pools with continuous exchange of water, maintaining natural temperatures and daylight.

Samples of phytoplankton, chlorophyll *a* and nutrients were taken on critical days of phytoplankton growth (initial day, during exponential growth-phase, at the beginning of stationary phase and at the end of stationary phase). Phytoplankton samples were preserved with acid Lugol's solution and cell counting was performed with a Leica inverted microscope according to the method of Utermöhl (1958). Samples of 25 ml were allowed to settle for 24 hours. At least 50 cells were counted for dominant species. Altogether at least 400 cells were counted per sample. The dimensions of 20 cells were measured for calculations of cell volume according to the Baltic Marine Biologists' recommendations (Edler, 1979). Dinoflagellate growth rates were calculated by $\mu = \ln(N_{max}/N_{min})/t$, where N_{max} and N_{min} are the maximal and minimal cell numbers and t is the time (days).

Chlorophyll *a* was analysed following the method of Jespersen & Christoffersen (1987).

Analysis of variance was used to test for significant differences between dinoflagellate growth rates in different treatments, as well as to compare the species diversity between treatments.

Table 1. Nutrient additions in the experimental treatments.

Days	Control	+DOM	+DOM+NO_3		+DOM+PO_4		+NO_3+PO_4	
		N-DOM	N-DOM	N-NO_3	N-DOM	P-PO_4	N-NO_3	P-PO_4
0	0	3.0	1.5	1.5	3.0	0.2	3.0	0.2
1	0	3.0	1.5	1.5	3.0	0.2	3.0	0.2
2	0	4.0	2.0	2.0	4.0	0.2	4.0	0.2
3	0	5.0	2.5	2.5	5.0	0.3	5.0	0.3
4	0	5.0	2.5	2.5	5.0	0.3	5.0	0.3
5	0	5.0	2.5	2.5	5.0	0.4	5.0	0.4
6–10	0	0	0	0	0	0	0	0
Total								
N (μM l^{-1})	0	25	25		25		25	
P (μM l^{-1})	0	0	0		1.6		1.6	

Nitrates and nitrites were analysed according to Wood et al. (1967). The methods of Koroleff (1983a, b) were used for estimation of concentrations of ammonia, phosphates, DON, DOP, and that of Koroleff (1976) for urea. Dissolved free and combined amino acids (DFAA and DCAA) were analysed as described by Mopper and Lindroth (1982) and Petty et al. (1982). Dissolved organic carbon (DOC) concentrations were determined by UV absorption (Pages & Gadel, 1990).

Results

Nutrient concentrations

Initial concentrations of inorganic nutrients were (in St. 121 and St. 34a, respectively): 0.12 and 0.15 μM l^{-1} NO_3, 1.52 and 1.28 μM l^{-1} NH_4, 0.17 and 0.06 μM l^{-1} PO_4. The concentrations of dissolved organic nitrogen (DON) were 19.0 and 13.1 μM l^{-1}, respectively. On Day 10, when the phytoplankton reached its maximum growth, nitrates were depleted by 100% in all treatments of both stations except in the +DOM+NO_3 treatment, probably due to lack of adequate P supply. Ammonium was depleted by 9–85% in St. 121, particularly in the Control treatment. In contrast, in St. 34a a decrease of ammonium concentrations occurred only in the +NO_3+PO_4 treatment, while accumulation of NH_4 occurred in others. Uptake of urea and DCAA was significantly higher in St. 121, where 16–40% of the total urea and 7–48% of the total DCAA was consumed. In St. 34a, uptake of urea occurred only in treatments +DOM+PO_4 and +NO_3+PO_4 (21 and 22%), while in the others accumulation was observed. DCAA concentrations decreased in samples from this station only by 4–26%. Accumulation of DFAA was observed in all treatments of both stations. Forty-four percent of the unidentified DON was consumed in treatment +DOM+NO_3 of St. 121, while release of DON was observed in others treatments for this station. In contrast, in St. 34a a decrease of the unidentified DON pool (23–38%) was recorded in all treatments where DOM was added. Inorganic phosphorus never became exhausted in both stations. In St. 121, PO_4 was taken up in all treatments by 41–97%, but in St. 34a by 31–96%. A decrease of DOP concentrations was observed in treatments +DOM and +DOM+NO3 (~5%) of St. 121 and in all treatments except +NO_3+PO_4 of St. 34a.

Phytoplankton biomass

The initial phytoplankton biomass of both sampling stations, in the central part of the Gulf of Riga (St. 121) and in the open Baltic Sea (St. 34a), was low (110 and 271 μg l^{-1} wet weight, respectively), as typical for the transient phase between spring and summer periods. Co-dominants in the initial phytoplankton community at St. 121 were cyanobacteria, chlorophytes and dinoflagellates, contributing 39%, 22% and 36% of the total biomass, respectively.

In St. 121, the growth of the phytoplankton community in the treatments +NO_3+PO_4 and +DOM +NO_3 started without the phase of adaptation, but in other treatments a decrease of phytoplankton biomass was observed on the Day 4 and a slow increase in later days of the experiment. At the end of exper-

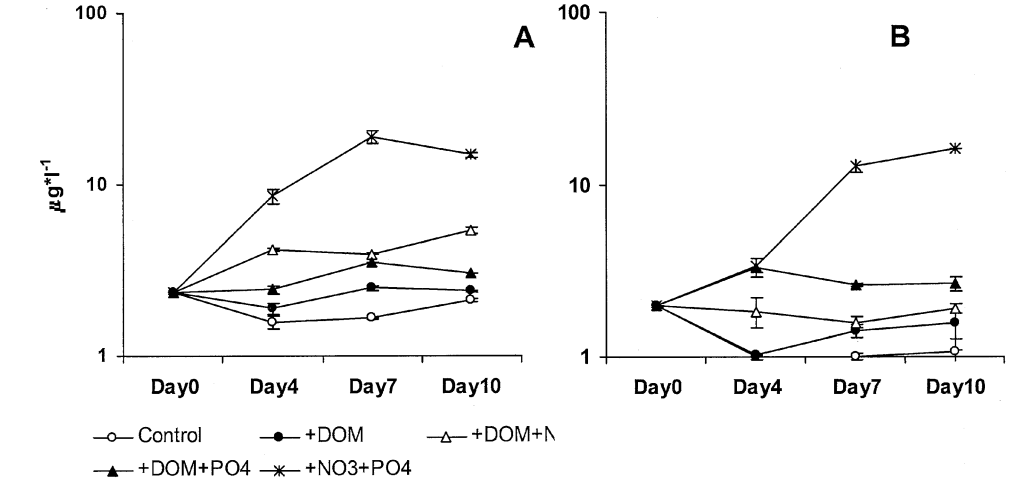

Figure 1. Chlorophyll *a* concentrations during a mesocosm experiment of the whole phytoplankton assemblage of St. 121 (A) and St. 34a (B), Mean ± SD, $n = 3$.

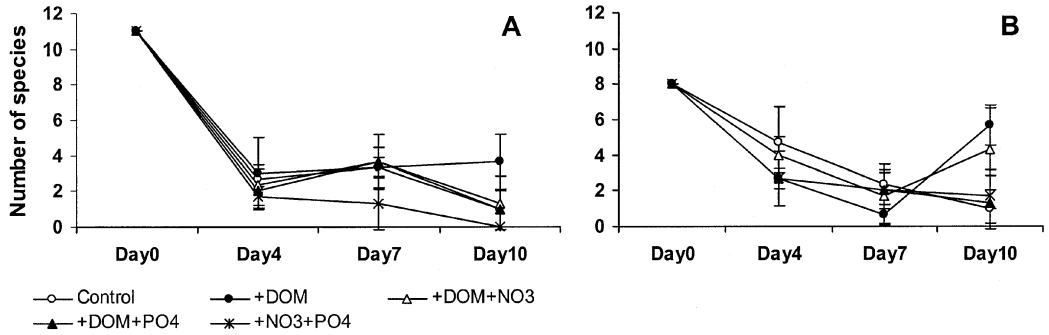

Figure 2. Number of dinoflagellates species in the nutrient addition treatments. Mean ± SD, $n = 3$.

iment, the highest abundance of phytoplankton and highest chlorophyll *a* concentrations were observed in treatment $+NO_3+PO_4$ ($p < 0.05$), followed by treatment $+DOM+NO_3$ (Fig. 1A), caused by increase of chlorophytes and non-filamentous cyanobacteria. Significant dinoflagellates growth was observed only in treatments +DOM ($p < 0.05$), where they increased from 27 $\mu g\, l^{-1}$ (initially) till 506 $\mu g\, l^{-1}$, contributing 27% of the total biomass at the end of experiment.

The initial phytoplankton community at St. 34a consisted of cyanobacteria (75% of the total biomass), chlorophytes and cryptophytes (~20% of the total biomass). Dinoflagellates contributed only 4.3% of the total biomass and even 17% of the total cell number. During the course of the experiment, the development of phytoplankton followed a similar pattern as in St. 121. The highest biomass and chlorophyll a was observed in treatment $+NO_3+PO_4$(Fig. 1B). Phytoplankton composition maintained the same proportions as in the initial community. However, an increase of dinoflagellates was observed in all treatments enriched with DOM. The highest biomass of dinoflagellates (569 $\mu g\, l^{-1}$, 60% of total biomass, $p < 0.05$) was observed in treatment +DOM, while in treatments $+DOM+NO_3$ and $+DOM+PO_4$ the biomass reached 143 and 183 $\mu g\, l^{-1}$, contributing ~28% of total biomass in both treatments.

Dinoflagellate growth rates

Altogether, 8 dinoflagellates species were identified at St. 121 (Table 2). The large-sized (35–45 µm) species *Dinophysis acuminata* dominated in the dinoflagellates assemblage (on average 1800 cells l^{-1}). The cell abundances of other dinoflagellate species were by an order of magnitude lower. At St. 34a eleven dinoflagellates species were recorded and *Katodinium rotundatum* (15–20 µm) was the dominant species.

Significant changes in dinoflagellate species composition were observed during the experiment. Ini-

Table 2. Initial phytoplankton community at St. 121 and St. 34a.

Group or species	Cell number (cells l^{-1})	Biomass ($\mu g\ l^{-1}$)
St. 121		
Bacillariophyceae	160	0.13
Chlorophyceae	127720	27.94
Cryptophcyeae	13470	4.84
Cyanophyceae	21440	49.53
Dinophyceae	2789	27.24
Total	165579	110.00
Dinoflagellates species:		
Amylax triacantha	20	0.20
Dinophysis acuminata	1800	23.59
Ebria tripartita	560	0.46
Gymnodinium albulum	20	0.01
Gymnodinium lohmannii	169	0.16
Peridiniella catenata	20	0.14
Peridinium baltica	80	0.92
Prorocentrum balticum	40	0.22
Protoperidinium brevipes	20	0.34
Protoperidinium divaricatum	40	0.80
Phalacroma rotundatum	20	0.40
Σ	2789	27.24
St. 34a		
Bacillariophyceae	640	0.82
Chlorophyceae	7860	18.08
Cryptophyceae	7860	3.63
Euglenophyceae	21890	32.83
Cyanophyceae	86540	203.48
Dinophyceae	25560	12.47
Total	150350	271.31
Dinoflagellates species:		
Dinophysis acuminata	120	1.57
Dinophysis norvegica	80	3.60
Ebria tripartita	560	0.46
Gymnodinium albulum	7860	3.14
Katodinium rotundatum	16840	2.11
Prorocentrum balticum	20	0.11
Protoperidinium brevipes	40	0.68
Protoperidinium divaricatum	40	0.80
Σ	25560	12.47

tially rich species diversity (8–11 species) sharply declined during the first 4 days of cultivation in all treatments (Fig. 2). *Ebria tripartita* disappeared completely, while *Heterocapsa triquetra* and *Peridinium baltica* were present in small quantities that they were rarely noticed during phytoplankton counts. On Days 7 and 10, a tendency of increasing dinoflagellate species diversity was observed. The highest number of species was recorded in treatments +DOM of both stations ($p < 0.05$).

In St. 121, the best growth was observed for *D. acuminata, Protoperidinium brevipes*, *Amylax triacantha* and *Gymnodinium albulum* (Figs. 3A–F). *D. acuminata* had the highest cell abundance in treatment +DOM, reaching 19800 ± 1235 cells l^{-1} ($P < 0.05$). This corresponds to a specific growth rate of 0.60 day^{-1} (Table 3). The next highest cell abundance of *D. acuminata* was observed in the Control (2443 ± 1225 cells l^{-1}), but in other treatments no significant changes in cell densities of *D. acuminata* were seen. A rapid increase of *D. acuminata* cells from Day 4 to Day 7 was observed only in the treatment +DOM+PO_4, with an estimated growth rate of 0.61 day^{-1}, but on Day 10 a decrease of cell densities was recorded.

An increase of *P. brevipes* cell density was observed in all treatments including the Control. A significantly higher growth rate was observed only in +DOM treatment ($p < 0.05$), compared to the other treatments (Fig. 3F). Other dinoflagellate species showed less remarked growth. Only *G. albulum* reached a high cell abundance and growth rate in the treatment +NO_3+PO_4. *A. triacantha*, *Peridiniella catenata* and *Prorocentrum balticum* gave preference to treatments enriched with DOM (Figs. 3B–E). On Day 10, significant increase of total dinoflagellates abundance occurred only in treatment +DOM (from 2789 to 25530 cells l^{-1}, corresponding to an increase of biomass from 27.34 to 423.70 $\mu g\ l^{-1}$, $P < 0.05$), in contrast to other treatments where dinoflagellate abundance increased only to 3430 cells l^{-1} (in treatment +DOM+NO_3) or even decreased to 600 and 1960 cells l^{-1} (in the Control and treatment +DOM+PO_4).

Changes in dinoflagellate species composition from the dominance of small-sized species versus large-sized species were observed in all the treatments of St. 34a. *K. rotundatum* cell densities sharply decreased from 16840 cells l^{-1} to ∼40 cells l^{-1} in all treatments while cell densities of *G. albulum,* another small-sized dinoflagellate, rapidly increased till the fourth day of cultivation and gradually decreased later (Figs. 4C, D). *D. acuminata* showed positive growth in all treatments, with the lowest increase in cell densities in the Control ($\mu = 0.08\ day^{-1}$), and the highest

Table 3. Mean growth rates (day^{-1}) for the dominant dinoflagellates species in the different nutrient treatments shown as mean ± SD. '–' denotes no positive growth rate or a decrease of cells numbers.

Station	St. 121					St. 34a				
Treatments	Control	+DOM	+DOM+NO_3	+DOM+PO_4	+NO_3+PO_4	Control	+DOM	+DOM+NO_3	+DOM+PO_4	+NO_3+PO_4
Dinophysis acuminata	0.26 ± 0.10	0.60 ± 0.05	0.28 ± 0.09	0.61 ± 0.08	0.06 ± 0.08	0.08 ± 0.02	0.59 ± 0.04	0.74 ± 0.06	0.80 ± 0.09	0.15 ± 0.05
Dinophysis norvegica						0.03	0.23	0.03	–	–
Ebria tripartita	–	–	–	–	–	0.17	–	0.1	0.1	–
Amylax triacantha	0	0.39 ± 0.01	0.26 ± 0.07	0.57 ± 0.03	–		0.49 ± 0.02			
Gymnodinium albulum	0.27 ± 0.02	–	–	0.84 ± 0.01	0.96 ± 0.01	0.36 ± 0.12	0.22 ± 0.10	0.40 ± 0.18	0.49 ± 0.12	0.20 ± 0.04
Katodinium rotundatum					–	–	–	–	–	–
Peridiniella catenata	0.52 ± 0.05	0.75 ± 0.02	–	–	–					
Prorocentrum balticum	0.15 ± 0.04	0.50 ± 0.02	0.57 ± 0.17	0.55 ± 0.07	0.50 ± 0.22	0.70 ± 0.08	0.40 ± 0.01	0.23 ± 0.11	0.4	0.31 ± 0.13
Protoperidinium brevipes	0.54 ± 0.10	0.71 ± 0.02	0.54 ± 0.06	0.69 ± 0.15	0.37 ± 0.08	0.06 ± 0.02	0.60 ± 0.01	0.45 ± 0.02	0.47 ± 0.04	0.38 ± 0.07
Protoperidinium divaricatum	–	0.07	–	–	–	–	–	–	–	–

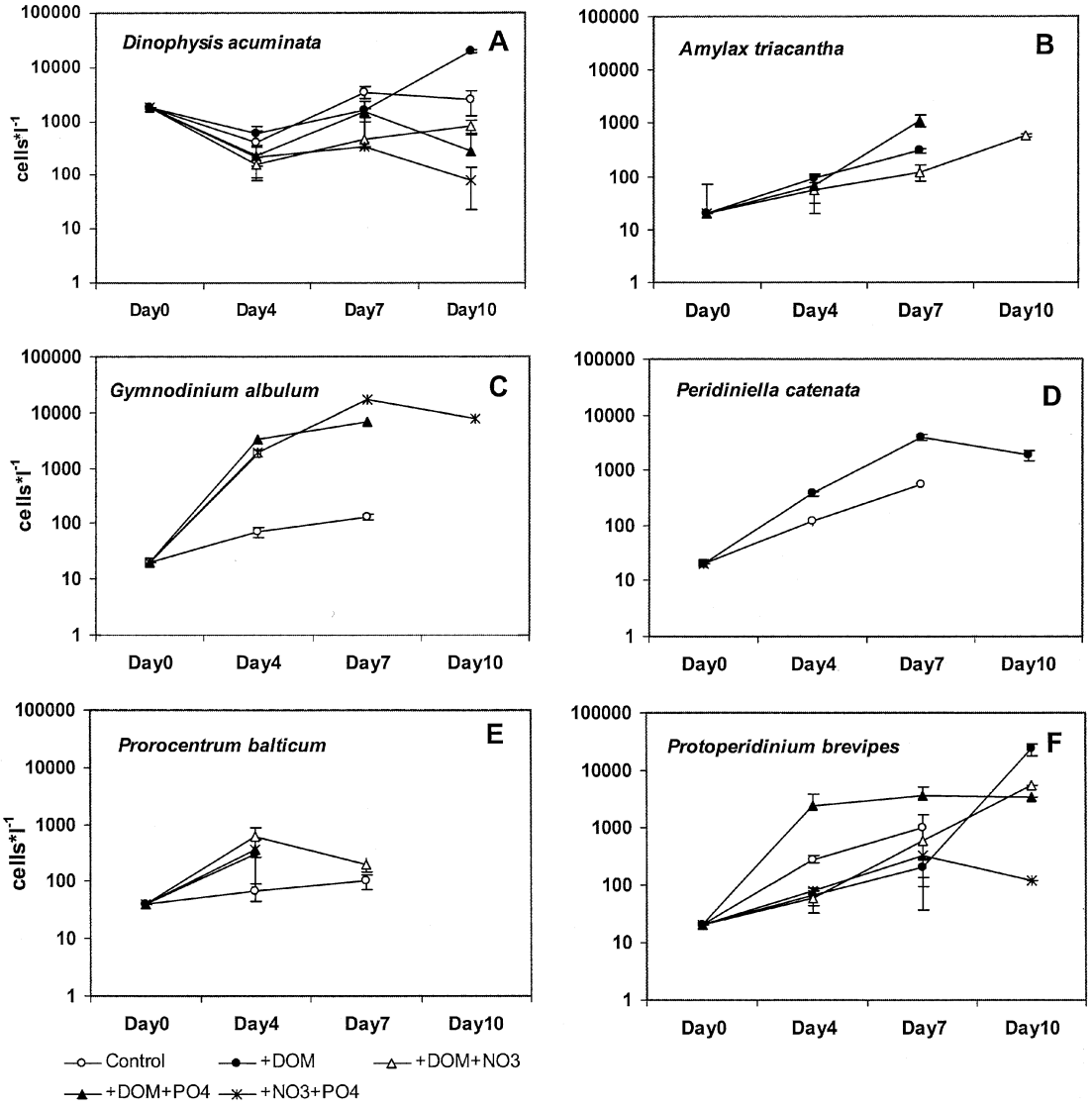

Figure 3. Development of the dominant dinoflagellates species in nutrient addition treatments of the mesocosm experiment at St. 121, Mean ± SD, $n = 3$.

growth in treatments +DOM+NO_3 and +DOM+PO_4 ($p < 0.05$), reaching densities of 7863 ± 1168 and 11557 ± 4887 cells l^{-1}, respectively, corresponding to specific growth rates of 0.74 day^{-1} and 0.80 day^{-1} (Table 3, Fig. 4A). *P. brevipes* had elevated densities in all treatments except the Control, with the highest cell density in treatment +DOM ($p < 0.05$). At the end of the experiment, an increase of total dinoflagellates biomass from 2789 initially to 14016 and 22805 cells l^{-1} was recorded in treatments +DOM+NO_3 and +DOM+PO_4, respectively, where *D. acuminata* composed 56 and 51% of dinoflagellate cell concentrations, but differences between both treatments were not statistically different. In treatment +DOM, the final cell concentrations were 34320 cells $l^{-1}$of which 46% were *P. brevipes*.

In treatments where *D. acuminata* had the highest growth rates the cells changed in external appearance, becoming opaque, filled with dark grains, as opposed to the typical transparent with clearly visible intracellular content. The same pattern was observed in all cases of massive development of *D. acuminata.*

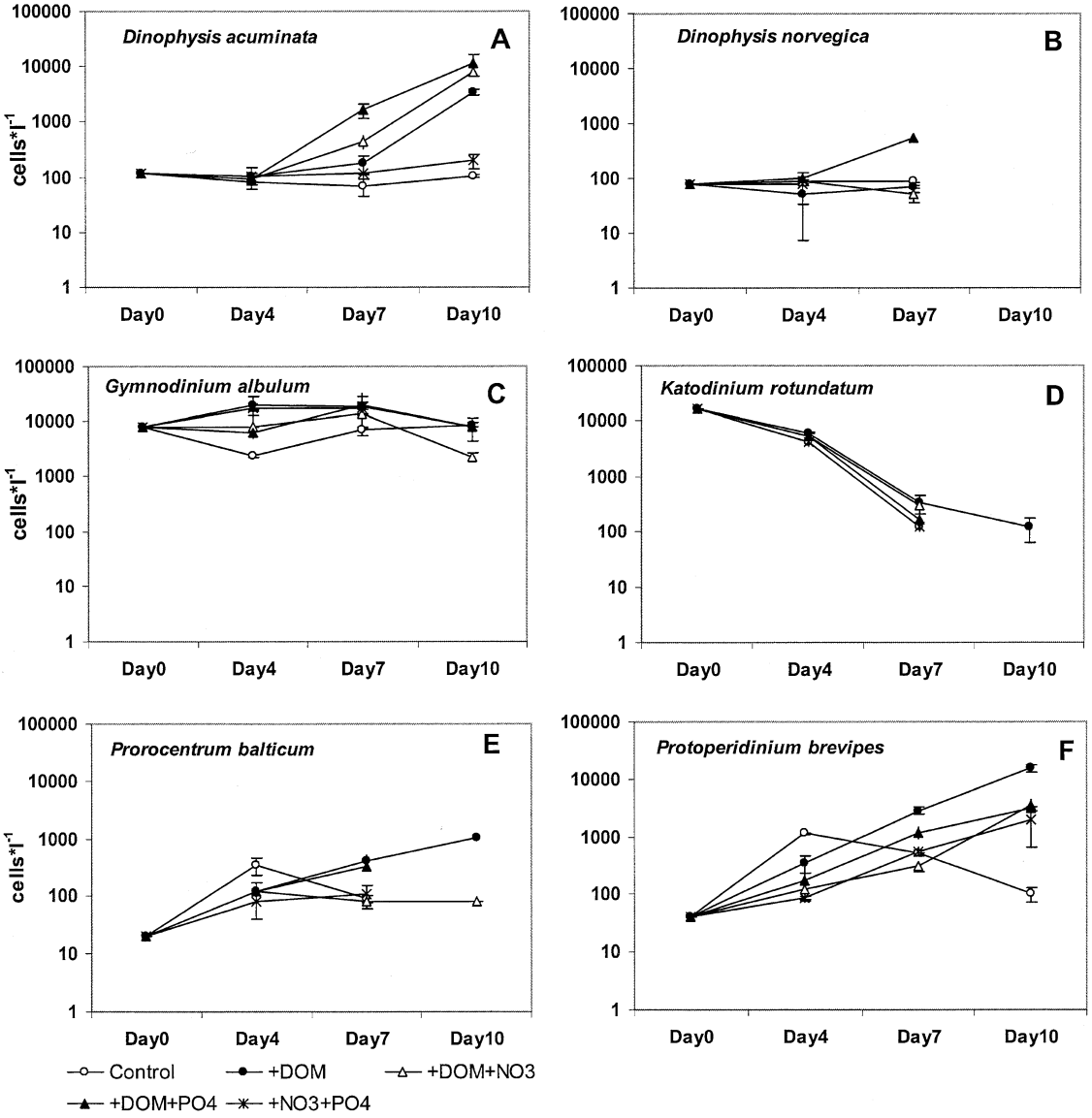

Figure 4. Development of the dominant dinoflagellates species in nutrient addition treatments of the mesocosm experiment at St. 34a, Mean $\pm$ SD, $n = 3$.

Discussion

The development of the phytoplankton community of St. 121 indicated nitrogen limitation, as the initial DIN: DIP ratio was ~10:1 and the highest biomass of phytoplankton was observed in treatments with higher concentrations of inorganic nitrogen ($+NO_3+PO_4$ and $+DOM+NO_3$). This observation was confirmed by the depletion of the dissolved inorganic nitrogen (DIN) in all treatments. In contrast, the DIN: DIP ratio at St. 34a was 24:1, suggesting importance of phosphorus for the development of the phytoplankton community. Thus, non-nitrogen fixing cyanobacteria and chlorophytes of both stations seemed to profit mainly from the inorganic nutrients, while a significant dinoflagellates growth was observed only in treatments enriched with DOM.

The initial species composition of dinoflagellates significantly differed between the stations. The large (35–45 μm) *D. acuminata* and *P. brevipes* dominated in the St. 121 and the small *K. rotundatum* (15–20 μm) in St. 34a. During the experiment, *D. acuminata* and

P. brevipes exhibited the best growth in both stations and dominated the dinoflagellates assemblage at the end of experiment. However, the factors promoting the development are thought to differ between species. *P. brevipes* is recognised as a phagotrophic species (Hansen, 1991; Larsen & Sournia, 1991; Barlewska & Witek, 1995; Latz & Jeong, 1996), etc.). The rapid decrease of the small-sized dinoflagellates *K. rotundatum* and *G. albulum* on Days 7 and 10 coincided with increasing densities of *P. brevipes*. Although the aspect of phagotrophy was not studied in our experiments, phagotrophy and availability of food resources might be the best explanation for the increase of *P. brevipes* in all treatments. Jeong (1994) reported an increased growth rate of phagotrophic dinoflagellates with increasing prey concentrations. They estimated the maximum growth rate for *Protoperidinium divergens* of 0.48 day^{-1}, while in our experiment the growth rates for *P. brevipes* ranged between 0.37 day^{-1} and 0.71 day^{-1}, thus significantly higher than in previous study. In our study, the highest growth rates of *P. brevipes* were observed in treatments enriched with DOM, where also other dinoflagellates had higher growth rates, accordingly prey become more available.

We could not clearly identify the factors promoting the growth of *D. acuminata*. This species is photosynthetic with functional chloroplasts. There are numerous studies showing the photosynthetic capacity of *D. acuminata*. Berland et al. (1994) estimated maximum division rate of 0.35 divisions day^{-1} ($\mu = 0.24$ day^{-1}) for *D. acuminata* in autotrophic conditions. Granéli et al. (1997) measured the photosynthetic carbon uptake rate of *D. acuminata* and other *Dinophysis* species and concluded that their growth rates in the light are comparable to other photosynthetic dinoflagellates. However, sometimes they observed significantly higher carbon uptake in the dark, indicating that mixotrophy could be induced when photosynthesis is not able to meet the requirements of the cells. Furthermore, Jacobson & Anderson (1996) used electron microscopy and demonstrated the occurrence of food vacuoles in *D. acuminata* and *D. norvegica*. All of these studies suggest that there exist several nutritional mechanisms for *D. acuminata* that can be induced probably by short-term favourable conditions or depending on previous adaptation. In our experiment we recorded a maximum growth rate of 0.74 to 0.80 day^{-1} which far exceeds previously reported growth rates for *D. acuminata.* The unusual external appearance of cells suggests that they could use also an untypical mode of nutrition.

Previous studies investigating cells physiology usually utilise monospecific cultures or concentrated/enriched natural communities (in case of *Dinophysis* spp.), which hinders the comparison with growth of a natural community where species competition can be just as important as nutrient additions. Garcés & Masó (2001) reported variability by a factor of 1.5 of *in situ* growth rates for different dinoflagellates species, showing that growth rates reflect a response of organisms to the environment. Incubations of high cell densities directly influence growth conditions and can produce an effect of no growth, while the natural population would be dividing actively (Agusti & Kalff, 1989).

In experiments with natural phytoplankton communities Carlsson et al. (1995) observed stimulation of dinoflagellate growth (small-sized *Katodinium* sp., *Gymnodinium* sp., *Amphidinium* sp. and large-sized *Gyrodinium* sp., *Scripsiella* sp., *Protoperidinium* sp.) in the presence of humic substances. Legrand & Carlsson (1998) observed significantly enhanced uptake of high molecular weight dextrans by *Alexandrium catenella* via pinocytosis in the presence of humic substances, independently from presence or absence of bacteria. They concluded that stimulating effect was due to previous adaptation of *A. catenella* to an organic medium. Our experiments showed that addition of DOM stimulated the growth of dinoflagellates, but the mechanisms still remain unclear. A drawback of experiments with natural phytoplankton communities is the inability to attribute the utilisation of some compounds to one group of organisms or one species. As we observed a decrease of dissolved organic compounds concentrations- such as urea, DCAA and the fraction of unknown DON, besides direct heterotrophic utilisation, we can suggest several other hypotheses. Berman et al. (1999) showed the release of NH_4^+ and urea from the natural DON pool, and a release of several amino and nucleic acids by bacteria and/or free dissolved enzymes. The authors suggested that this could be the major pathway of N flux from the DON pool to phytoplankton (and bacteria) in environments with low concentrations of dissolved inorganic nitrogen.

Another hypothesis is that DOM can alter the underwater light climate by reducing photosynthetically active radiation (reviewed by Doblin et al., 1999). Tang (1996) suggests that dinoflagellates have a lower chlorophyll *a*/carbon ratio than other algal groups of

the same size and consequently lower photosynthetic abilities. It has been shown that dinoflagellates also have a lower light saturation level (Chang & Mcclean, 1997). This is not surprising considering that there are known only six strictly autotrophic dinoflagellates species (Gaines & Elbrächter, 1987). Probably, dinoflagellates can compensate for this by feeding heterotrophically (Tang, 1996) or even, the ability to live in the environment with reduced PAR may provide dinoflagellates with an advantage in competition with other species.

We found a significantly higher species diversity of dinoflagellates in the treatments with DOM. This can be explained by the complex chemical structure of DOM. Dissolved organic matter of terrestrial origin is composed of polypeptides, polysaccharides, polyphenolic humic substances and also dissolved amino acids, nucleic acids and vitamins. This provides a wide array of substrates for growth of different algal species and other organisms, while addition of NO_3 leads to dominance of specific species.

It is possible that in nature many mechanisms can function simultaneously while in experimental conditions usually one mechanism dominates. We believe that heterotrophic utilisation of dissolved organic compounds at lower light intensities could be the best explanation for the enhanced growth of the dinoflagellate population. However, bacterial degradation of DON to simple reduced N forms like ammonium and urea could be the major source of nitrogen for other phytoplankton groups when amounts of inorganic nutrients are limited.

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Temporal and spatial patterns of crustacean zooplankton dynamics in a transitional lagoon ecosystem

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Key words: zooplankton, seasonal dynamics, water residence time, eutrophic lagoon

Abstract

Patterns and mechanisms of plankton crustacean seasonal succession in the eutrophic freshwater Curonian lagoon (south-eastern Baltic Sea) were analysed on the basis of four-year (1995, 1996, 1998 and 1999) field sampling results. The seasonal crustacean zooplankton succession in the lagoon appears to be the consistent six-stage sequence of four distinct species complexes. Each stage is characterised by its individual species composition and quantitative characteristics. The uniform and periodic pattern of the limnetic zooplankton crustacean successional stages in the lagoon indicates that the seasonal succession of the limnetic zooplankton is not disturbed by unpredictable environmental fluctuations, such as brackish water inflows. Seasonal zooplankton succession is also comparatively uniform at a spatial scale. Not more than two adjacent successional stages were found across the northern part of the lagoon during each of 11 seasonal surveys. Comparison between monthly water residence time and dominant plankton crustacean species life cycle duration points to a more transitory plankton community in spring while in the summer it is not much influenced by lagoon hydrodynamics. Consequently, the Curonian lagoon crustacean community quite closely follows the Plankton Ecology Group (PEG)-described freshwater lake seasonal succession in summer and turns into a lentic-like system in spring and autumn.

Introduction

Mechanisms of plankton seasonal succession have been investigated for years, resulting in a number of concepts and theories (Brooks & Dodson, 1965; Carpenter & Kitchel, 1984, 1996; Persson, 1999). One of the most successful attempts to integrate different concepts is the PEG model (Sommer et al., 1986), explaining freshwater plankton seasonal succession in the idealised temperate lake. Analysis of plankton dynamics in physically extremely variable estuarine and lagoon environments, coupled altogether with a wide variety of hydrochemical conditions is, however, far more difficult. The interplay of strong gradients in river flow, mixing, nutrient inputs, salinity and temperature produces spatially and temporally heterogeneous conditions for plankton communities (e.g. McLusky, 1981, 1993; Laprise & Dodson, 1993, 1994). Therefore, they are mainly 'physically controlled' in opposition to 'biologically accommodated' limnic communities (Day et al., 1989). Main differences in regulatory mechanisms between lakes and freshwater estuaries may correspond to a different level and importance of such external forcing as hydraulic regime and hydrochemical variability.

In the Curonian lagoon only phytoplankton seasonal succession (Olenina, 1997, 1998) has previously been investigated. Our purpose was to delineate patterns and mechanisms of plankton crustacean seasonal succession in the eutrophic freshwater lagoon as well as to compare it with the classic PEG model sequence.

Materials and methods

Study site

The Curonian lagoon (south-eastern Baltic Sea; Fig. 1) is mostly freshwater and only in the northern part (directly connected to the Baltic Sea) the salinity varies,

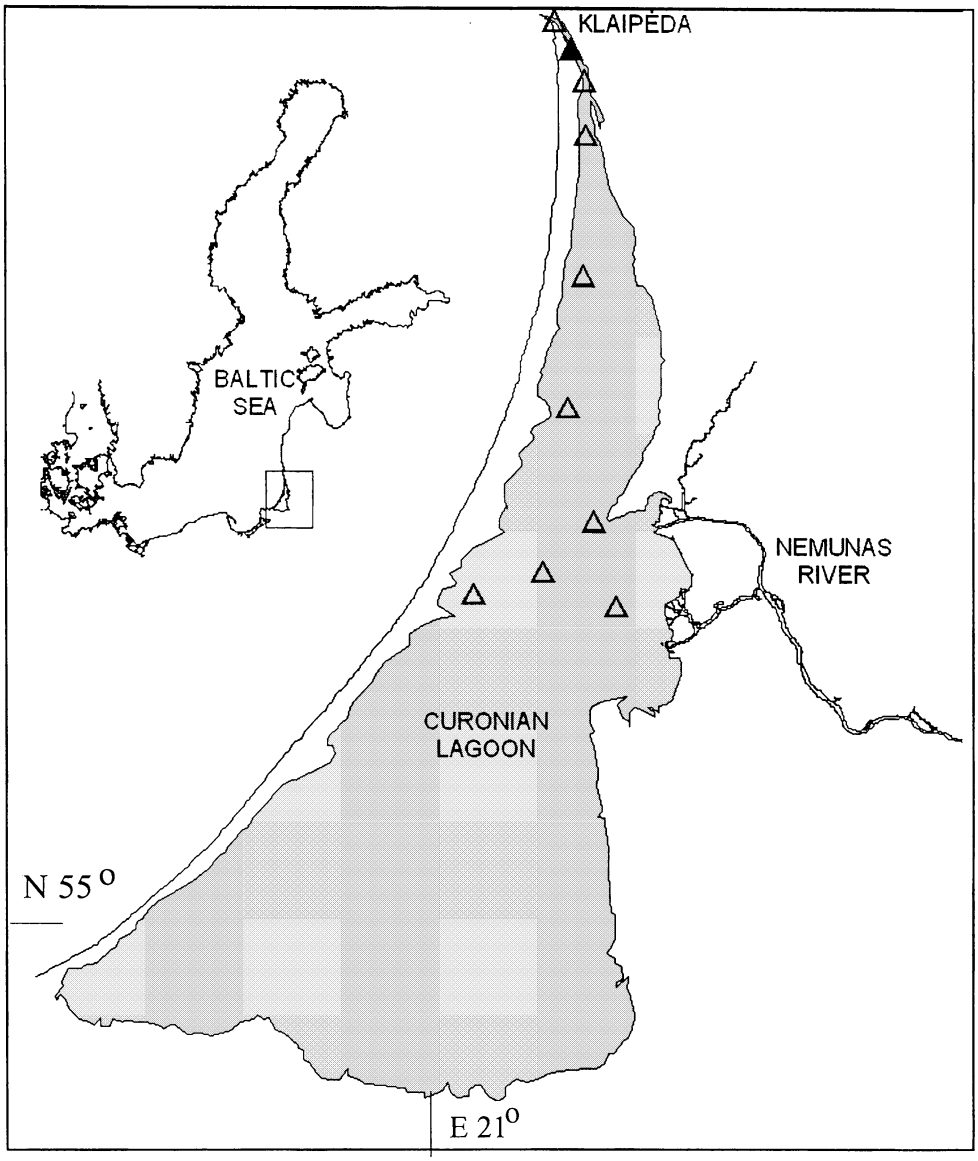

Figure 1. Study area. Filled triangle (A) long-term seasonal monitoring site, open triangles (B) seasonal survey sites.

from 0 up to nearly 8 psu and the average annual salinity is 3.6 psu (Žaromskis, 1996; Dubra, 1996). On average, 23 km^3 of the river water pass the Klaipėda strait per year and 5 km^3 enters with the Baltic Sea water inflows (Dubra, 1996). Duration and extent of the seawater intrusions depend on the wind-induced rise of water level in the coastal zone of the sea (Žaromskis, 1996). Usually, the residence time of the brackish water in the lagoon is not longer than 5 days. Most common are 1–2 day inflows of Baltic Sea water. Water temperature shows a characteristic temperate pattern ranging from 0.1–0.2 °C in winter to 5–15 °C in spring and reaching the highest values (up to 19.1–19.3 °C) in July–August (Žaromskis, 1996; Pustelnikovas, 1998).

The phytoplankton community structure and dynamics in the Curonian lagoon exhibits a pattern typical of eutrophic water bodies. The diatom bloom occurs in April–May and is dominated by *Stephanodiscus hantzschii*. Cyanobacteria development (mostly *Aphanizomenon flos-aquae*) starts in the end of June, reaching maximum abundance at temperatures above 20 °C (Olenina, 1998). There are no planktivorous fish in the lagoon, except juveniles, normally restricted to

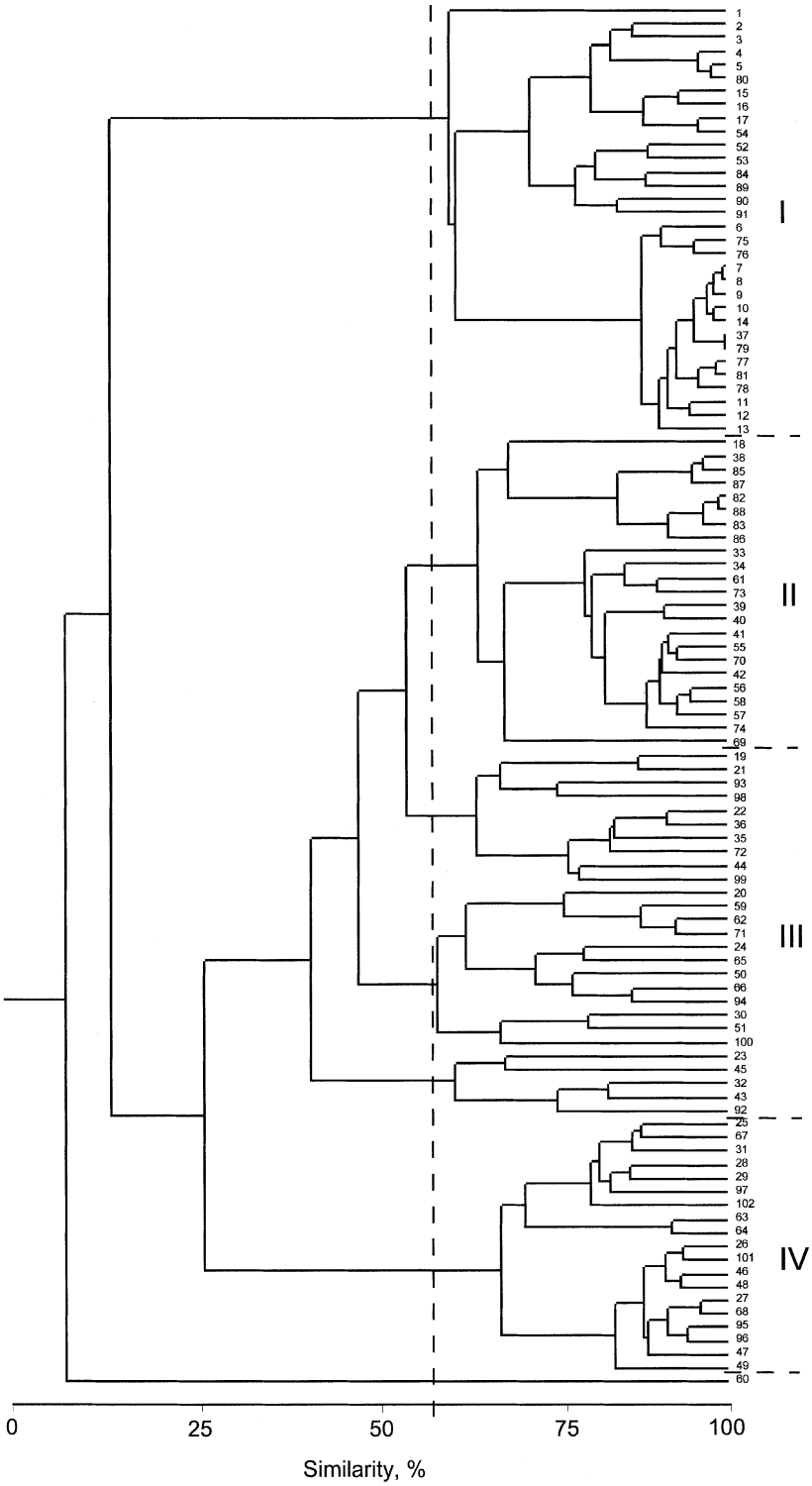

Figure 2. Dendrogram of hierarchical clustering (group average linking) of 102 seasonal zooplankton samples based on Bray–Curtis similarity. The 60% similarity level and four corresponding groups of samples are marked.

the littoral zone. Invertebrate predators are represented by *Leptodora kindti*.

Sampling and sample treatment

Zooplankton was sampled at a single station in the northern part of the Curonian lagoon (Fig. 1A), approximately every 3 to 5 days from February to November in 1995 and 1999, April to October in 1996 and March to October in 1998 (174 samples). Eight to nine stations were sampled 1–2 times per month during May–August in 1995–1996 (97 samples in total, Fig. 1B). Pooled duplicate vertical hauls were taken with a 100 μm mesh Juday plankton net (13 cm in diameter). Organisms were preserved in 4% formaldehyde. Temperature and salinity were measured at each sampling occasion. All samples were taken in the pelagic zone of the lagoon to avoid spatial heterogeneity associated with sampling from different habitats.

Planktonic crustaceans were identified to the species or genus level (Rylov, 1948; Flosner, 1972; Tsalolikhin, 1995). *Daphnia* specimens, belonging to '*longispina*' complex were analysed as a single taxon. At least 50, but generally more than 100 individuals of the most abundant zooplankton species excluding copepod nauplii were counted, giving a 20% error, which is considered appropriate for zooplankton analysis (Kozhova & Melnik, 1978).

Data analysis

The general pattern of zooplankton seasonal succession was derived using the samples taken in the northern part of the lagoon (Fig. 1A). Since stochastic brackish water inflows could bias freshwater community characteristics, the samples taken at salinity >0.5 psu (34% of the samples) were excluded from the study.

Species comprising more than 10% of the total zooplankton density were defined as dominants. To obtain a more holistic picture of zooplankton succession, successional groups of crustaceans were derived instead of using separate species. The relative abundance values of seven dominant crustacean species [*Bosmina* spp. (*Bosmina coregoni* + *Bosmina longirostris*), *Daphnia 'longispina'*, *Chydorus sphaericus*, *Cyclops strenuus*, *Diaphanosoma brachyurum*, *Eudiaptomus graciloides*, *Mesocyclops leuckarti*] and one cumulative group containing the rest of the crustacean species were used for the analysis. Affinities between all seasonal samples were calculated to define the successional groups. Following the recommendations of Clarke & Warwick (1997), hierarchical clustering procedure with group-average linking based on Bray–Curtis similarity coefficient and ordination of samples by multi-dimensional scaling (MDS) were used.

The spatial pattern of the limnetic zooplankton seasonal succession was evaluated on the basis of periodic sampling at 8–9 fixed stations in 1995–1996. Only the samples taken at salinity <0.5 psu were considered. Each sample was classified to fit into one of the successional groups described above.

Mean monthly water residence time was calculated using the daily Nemunas river discharge observations in 1990–1997 (Lithuanian Ministry of Environment, unpublished monitoring data). Since all of the river discharge passes through the northern part of the lagoon, we calculated water residence time for this part separately, suggesting the southern part as being generally stagnant.

The approximate dominant crustacean species life cycle duration was calculated for each of the successional stages using literature data on crustacean development (Bottrell et al., 1976; Ghiliarov, 1987; Maier, 1994; Arbačiauskas & Gasiūnaitė, 1996) and temperature corrections (Khmeleva & Golubev, 1984).

Results

Successional groups of the limnetic plankton crustaceans

The dendrogram of sample similarity, calculated on the basis of seven dominant taxa (*Cyclops strenuus*, *Bosmina* spp., *Chydorus sphaericus*, *Daphnia 'longispina'*, *Diaphanosoma brachyurum*, *Eudiaptomus graciloides* and *Mesocyclops leuckarti*) and one cumulative group relative abundance, showed a clear separation into six clusters at approximately 60% similarity (Fig. 2). Three groups of the samples (clusters I, II and IV) were structurally most homogeneous. *C. strenuus* distinctly dominated in the samples of group I (Fig. 3). Within this group the samples fell into two smaller sub-clusters at 88% and 73% similarity. Dominance of *C. strenuus* (87–100% of total crustacean abundance) was characteristic for the first sub-cluster, whereas the second sub-cluster combined samples with higher dominance of other species such as *M. leuckarti*, *Bosmina* spp. and *D. 'longispina'* (up to 33%). No individuals of *D. brachyurum* and only single specimens of *C. sphaericus* were observed in the samples belonging to group I.

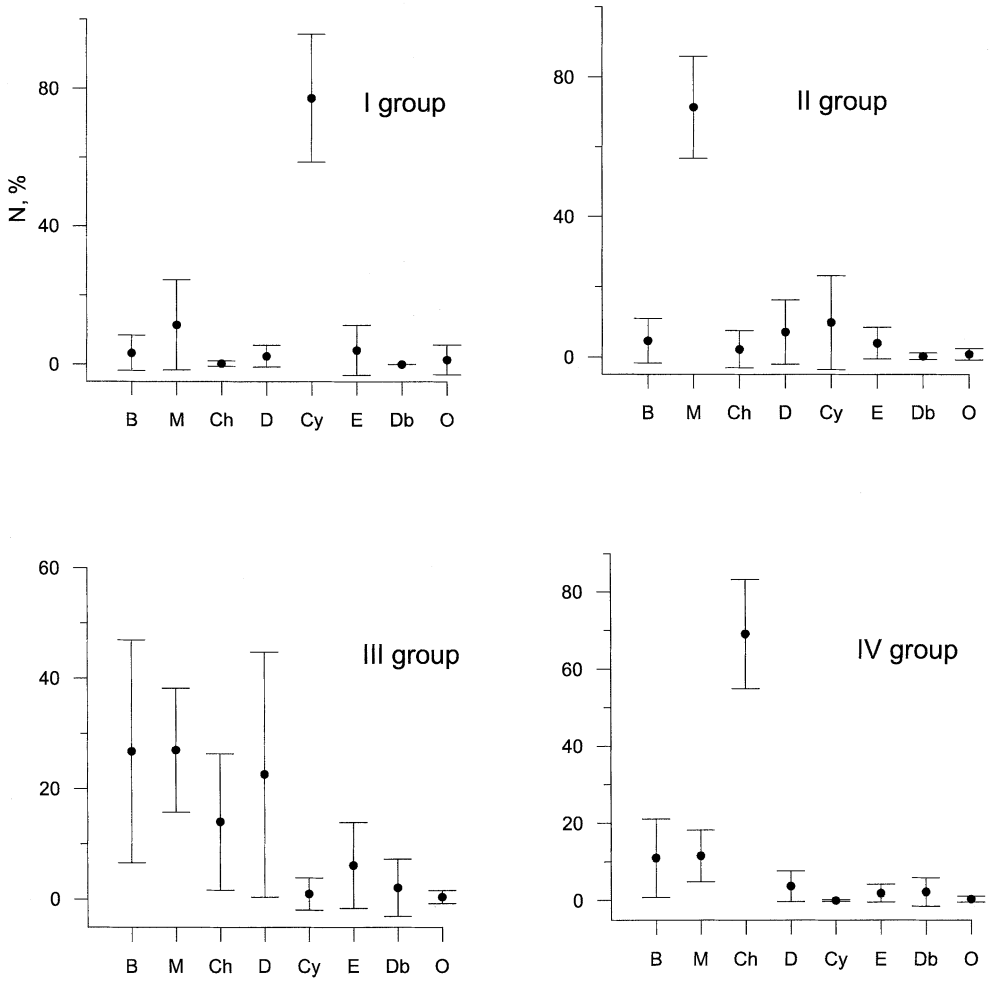

Figure 3. The relative density (N) of *Bosmina* spp. (B), *Mesocyclops leuckarti* (M), *Chydorus sphaericus* (Ch), *Daphnia 'longispina'* (D), *Cyclops strenuus* (Cy), *Eudiaptomus graciloides* (E), *Diaphanosoma brachyurum* (Db) and other crustacean species (O) in the four groups of samples derived from the clustering procedure (Fig. 2); $n = 32, 23, 27$ and 19 for group I, II, III and IV, respectively. Values are means $\pm$ 1 SD.

Groups II and IV were characterized by high relative abundances of *M. leuckarti* and *C. sphaericus*, respectively (Fig. 3). Comparatively high dominance of *C. strenuus* (up to 38%) was observed in several samples of group II. The samples belonging to group IV fell into two distinct sub-clusters at 85% and 72% similarity (Fig. 2), corresponding respectively to the highest dominance of *C. sphaericus* (74–97% of total abundance) and shared dominance between *C. sphaericus* (41–66%), *Bosmina* spp. (2–34%) and *M. leuckarti* (4–27%; Fig. 3).

Three smaller clusters were analysed as one heterogeneous group (III). No single species exceeded 60% of relative density in the samples of this group. Dominance was shared between *Bosmina* spp., *M. leuckarti*, *C. sphaericus* and *D. 'longispina'*. High variation of these species relative densities was characteristic for this group of samples (Fig. 3). Comparatively high relative abundances of *E. graciloides* (up to 25%) and *D. brachyurum* (up to 19%) were also detected.

The results of cluster analysis were similar to those in the multidimensional scaling plot (Fig. 4). The MDS plot shows quite tight grouping of the samples belonging to groups I, II and IV, derived by cluster analysis. The samples of group III are scattered between the II and IV groups, suggesting the transitional character of this group. According to the MDS plot, the sample 60, appearing as outlier in cluster analysis and characterised by unusually high dominance (95% of the total abundance) of *Bosmina* spp. could be attributed to the III group. In contradiction to the

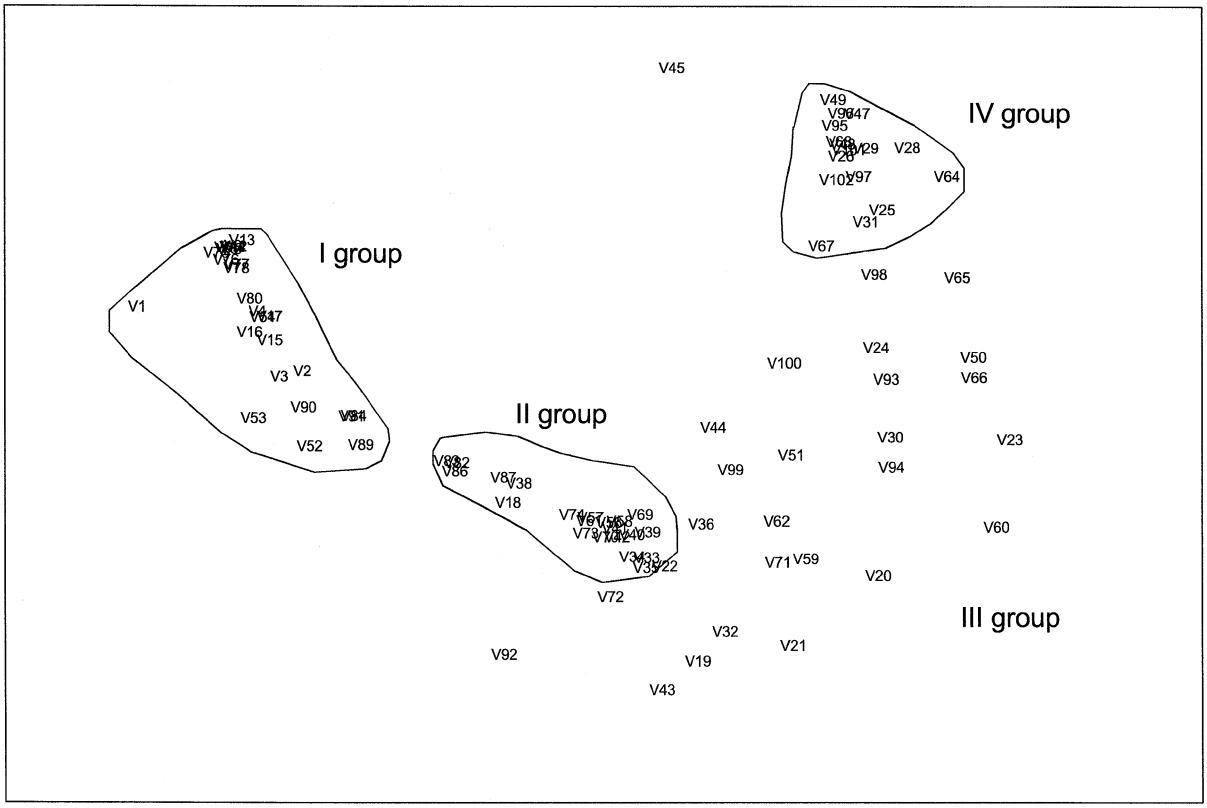

Figure 4. The MDS plot of 102 seasonal zooplankton samples; marked groups correspond to those derived by the cluster analysis (Fig. 2).

results of cluster analysis, samples 22 and 35 were ascribed to II group.

Stages of the zooplankton seasonal succession

All four groups of samples derived from both clustering and multidimensional scaling techniques were placed along the temporal axis and formed a continuous sequence of six seasonal succession stages (Fig. 5).

All samples of group I corresponded to the first stage, related to the lowest mean water temperature and were characterised by a strong dominance of the large copepod *C. strenuus*. The share of *M. leuckarti* was insignificant during the end of winter–early spring, while it reached up to 30% at the end of April–May period.

The prevalence of *M. leuckarti* was distinctive for the second stage of seasonal succession. The share of the cladocerans *Bosmina* spp. and *D. 'longispina'* reached up to 26 and 21%, respectively, in the end of May–beginning of June.

Dominance was almost equally shared between three herbivorous cladocerans and one copepod species at the structurally most heterogeneous third stage. The relative abundance of small *Bosmina* and *Chydorus* species reached up to 62% and 34%, respectively.

The fourth stage coincided with the highest mean water temperature (20.5 °C). Compared to other stages, the lowest variation in timing of the fourth stage was due to *C. sphaericus* population dynamics, characterised by steep density peaks during all four years of study.

No distinct dominant species were observed during the fifth stage, while the sixth stage was strongly dominated by *M. leuckarti*. These two stages corresponded to the II and III groups of samples, respectively.

Spatial heterogeneity of the zooplankton succession

The seasonal succession showed a spatially recurrent pattern during the research cruises in 1995 and 1996 (Fig. 6). The timing of the stages coincided with that proposed in Fig. 5. In five cases, the single

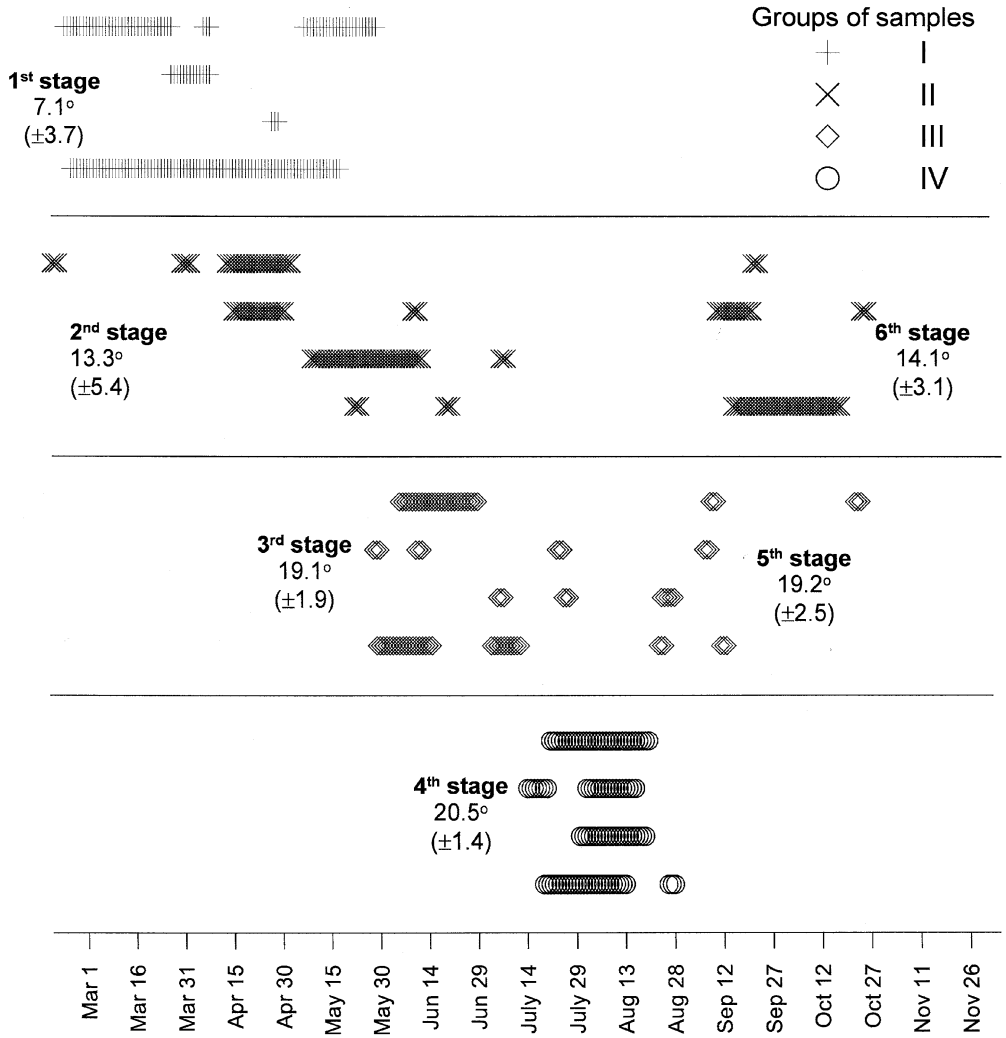

Figure 5. The sequence of zooplankton seasonal succession in the Curonian lagoon. Data representing the years 1995, 1996, 1998 and 1999 for each stage are arranged from bottom to top. Water temperatures are given as mean values ± 1 SD.

successional stage was observed throughout the lagoon. The crustacean community structure appeared to be rather stable on 9 May 1995 and 11 June 1996; the early successional stages (first and second stages) were prevalent on both occasions.

In other cases, the variation of structural characteristics between stations was more pronounced. However, no more than two adjacent successional stages were found during each simultaneous sampling event.

Discussion

Following the PEG scheme, zooplankton community structure in eutrophic waters generally shifts from large herbivore dominance in spring towards the dominance of smaller species during summer (Sommer et al., 1986). However, in the Curonian lagoon, predatory cyclopoids dominated in spring, while large herbivorous *Daphnia* species appeared in the beginning of summer. A PEG-like shift to smaller herbivores was observed later in midsummer. This is commonly related to the occurrence of cyanobacteria colonies, which are too large to ingest, low in nutritional value or even toxic (DeMott, 1989). Larger Cladocera species are usually most negatively affected by cyanobacteria (e.g. Hawkins & Lampert, 1989), while small species are able to avoid the blocking of the filtering apparatus (DeMott, 1989). Thus, the development of large filamentous *Aphanizomenon* colonies

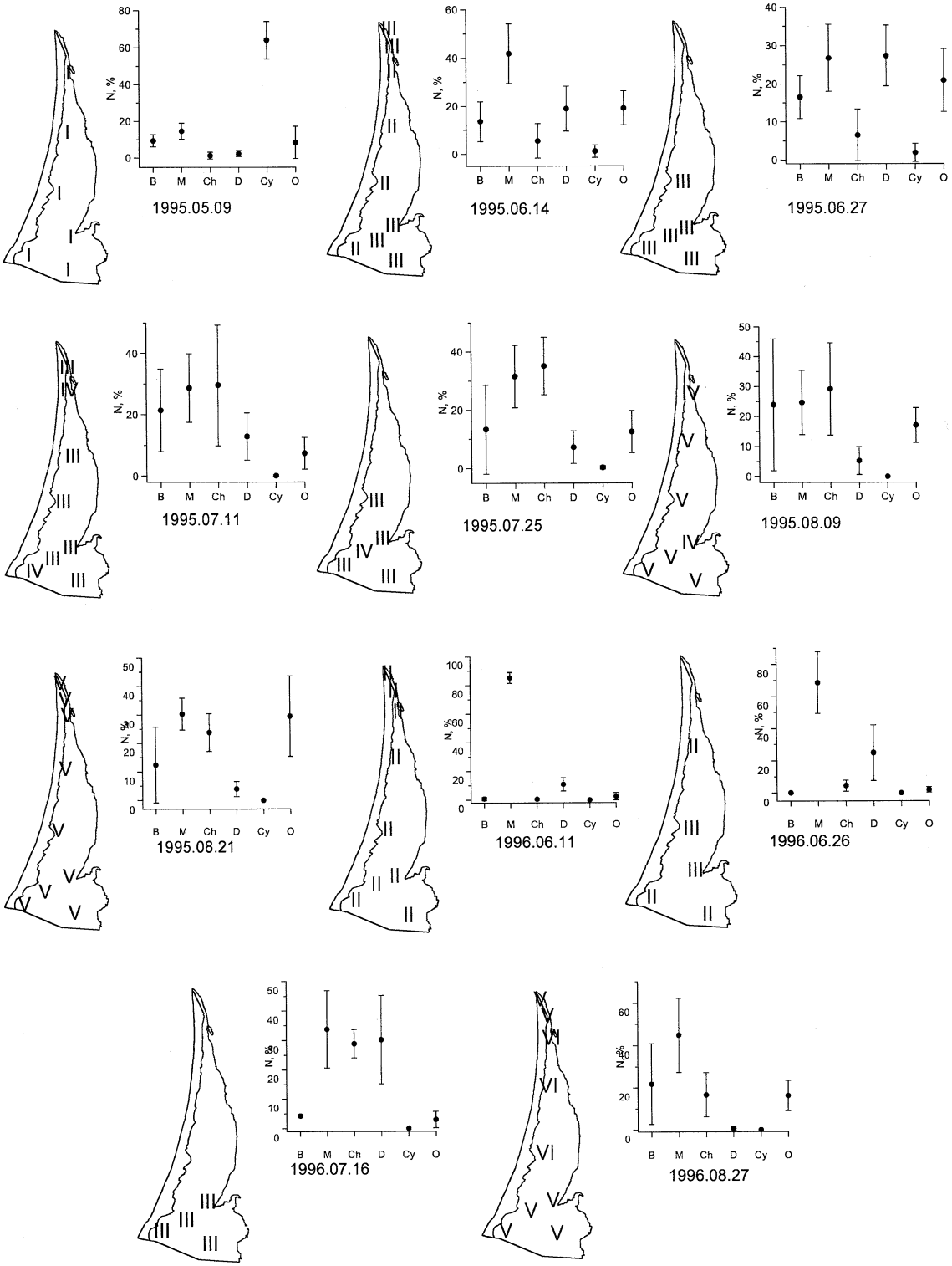

Figure 6. The spatial pattern of zooplankton seasonal succession in the Curonian lagoon. The seasonal succession stages are indicated for each sampling site. Graphs show the average crustacean community structure; abbreviations as in Fig. 3.

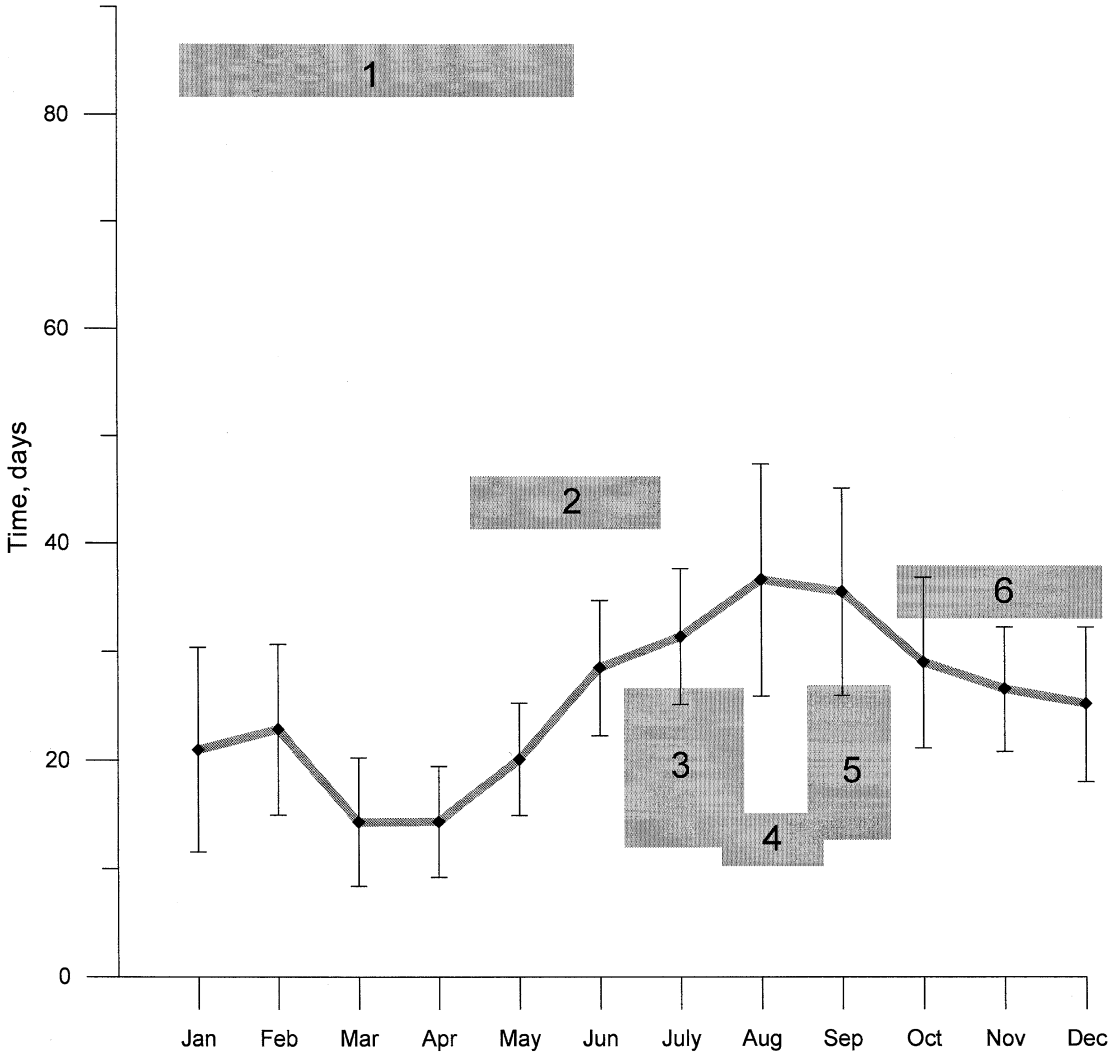

Figure 7. Mean monthly water residence time (solid line) ± 1 SD in the northern part of the Curonian lagoon, based on daily discharge observations in 1990–1997. Successional stages are indicated as grey rectangles. The rectangle width represents successional stage duration, height represents variability in dominant crustacean species life cycle duration.

in the Curonian lagoon as reported by Olenina (1997, 1998) may be considered as an important factor for zooplankton species succession in summer. Our results on autumn crustacean zooplankton succession in the northern part of the Curonian lagoon are rather complicated due to increasing frequency and duration of the brackish-water inflows. Nevertheless, the observed return to the Cyclopoida-dominated community in September–October is contrary to the PEG model (Sommer et al., 1986) which suggests autumn development of large herbivores.

The major sources of deviations from the PEG model of seasonal plankton succession in both oligotrophic and eutrophic waters are supposed to be high flushing rates, lack of thermal stratification and shallowness. These factors could cause accidental events in plankton communities, unpredictability and break of endogenous succession (Sommer et al., 1986). Despite the shallowness of the Curonian lagoon, absence of thermal stratification (Pustelnikovas, 1998) and stochastic brackish water inflows may temporarily change zooplankton community structure and abundance in the salinity gradient zone (Gasiūnaitė, 2000), summer zooplankton succession in the Curonian lagoon follows the PEG model quite closely. Spring and autumn cyclopoid dominated communities are, how-

ever, totally different from the ones suggested in the eutrophied lakes by PEG model.

Seasonal zooplankton succession was comparatively uniform at a spatial scale. Not more than two adjacent successional stages were found in the lagoon during each sampling event (Fig. 6).

Differences between the classic PEG model and the zooplankton seasonal succession in the Curonian lagoon could be explained by comparing the functioning of limnic and estuarine systems. In transitory (riverine and estuarine) ecosystems, zooplankton as well as whole plankton communities, can be influenced and even controlled (Mortazavi et al., 2000) by passive drift to the sea and brackish water intrusions causing spatial and temporal salinity gradients. The Nemunas river outflow, expressed as monthly water residence times, appeared to have a clear seasonal pattern being shortest during the spring flood season and longest in summer (Fig. 7). Welker & Walz (1999) reported that a water residence time longer than 8 days is preferable for plankton development in transitory water systems dominated by rotifers, which is roughly two times longer than average rotifer generation time (Bottrell et al., 1976; Wetzel, 1983; Jorgensen et al., 1995). Life cycle duration or doubling time in relation to residence time could be used to describe preference limits for particular species (Cloern et al., 1985). Simple comparison of dominant plankton crustacean species life cycle duration to water residence times in the northern part of the Curonian lagoon points to a more transitory plankton community in spring, while in summer it could hardly be influenced by the lagoon hydrodynamics.

The latest Nemunas River investigations (Gasiūnaitė & Pilkaitytė, 2001) revealed that the summer zooplankton community structure, featuring dominance of cyclopoid species and low cladoceran densities, is similar to the spring and autumn zooplankton communities in the lagoon (first, second and sixth stages, Fig. 5). These particular two stages of Curonian lagoon crustacean seasonal succession are not found in PEG model. Taking into account the similarity of hydrodynamic conditions between the Nemunas river in the summer and the Curonian lagoon in the spring and autumn flood periods, as well as the seasonal trends of dominant plankton crustacean life cycle duration, we suggest hydrodynamic forcing to be responsible for these discrepancies. Thus, Curonian lagoon zooplankton community quite closely follows PEG-described freshwater lake seasonal succession in summer and turns into a lentic-like system in spring and autumn.

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Classification and description of phytobenthic communities in the waters of the West-Estonian Archipelago Sea

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Key words: classification, phytobenthos, West-Estonian Archipelago, Baltic Sea

Abstract

Three separate coastal sea areas were investigated in terms of phytobenthic community structure in the waters of the West-Estonian Archipelago in the Baltic Sea. The phytobenthos species composition was similar in the three regions while the structure of the communities referred to different environmental conditions. The classification of the phytobenthic communities in the area was made using cluster analyses. The defined and described 19 classification units were compared with classification systems that have been published before. Most of the classification units defined in the present study were similar to the units defined by other authors. Depth and substrate had considerable influence on the phytobenthic communities in three areas: in the westernmost, exposed sea area phytobenthic communities changed continuously along the depth gradient without forming distinct belts while in the more sheltered central areas wide, depth-related phytobenthos belts were described.

Introduction

Estonian coastal areas are among the best studied for phytobenthic communities in the whole Baltic Sea. The most recent comprehensive investigations date back to 1970s and cover large territories within the area (Kukk, 1979; Trei, 1973, 1991). Taking into account the constantly changing environmental conditions due to local and Baltic Sea scale human induced eutrophication, as well as global climatic processes and the necessity of current updating of the scientific information, the present study was designed to describe the state of phytobenthic communities in three different sea areas in the coastal sea of the West-Estonian Archipelago.

The three goals of our study were (1) to describe the present state of the phytobenthos of three localities, (2) to develop a classification system for phytobenthic communities of the West-Estonian Archipelago sea area based on previous field studies and on mathematical classification techniques, and (3) to evaluate the role of different environmental factors on the structure of the benthic communities in the area.

The species composition of the phytobenthos in all three areas has been investigated in the past by different authors. Häyrén (1930, 1936) described the species composition of the phytobenthos in the Vilsandi area during his expeditions to the Estonian islands, including the western coast of Saaremaa island. Later, Trei (1973, 1987) described the macrophytobenthos of the whole West-Estonian Archipelago area including all three areas of the present investigation. Detailed species lists of phytobenthos of Vilsandi area was given by Kukk (1988). The species composition in the Hullo Bay on the southern coast of Vormsi island was described by Martin (1994).

The aim of establishing a proper classification of the phytobenthic communities is not the classification system itself. At present it is meant to be more a practical tool connected to the proper management of coastal marine ecosystems demanding large-scale habitat classification and mapping (HELCOM, 1998). Until today, few attempts have been made to classify phytobenthic communities of the northeast Baltic

proper (Kukk, 1979; Kautsky & van der Maarel, 1990; Trei 1991; Reitalu et al., 2002).

In the Baltic Sea, the main environmental variables structuring the phytobenthic communities in a given area are depth (as a function of light intensity and ice scraping), substrate type and wave exposure (Kautsky et al., 1986; Kautsky, 1988; Kautsky & van der Maarel, 1990; Kiirikki, 1996; Kiirikki & Ruuskanen, 1996). Other factors, which can influence the development of the benthic communities, have been discussed in the literature, such as salinity, ice scraping, temperature and biotic interactions (competition for space and nutrients and grazing).

Material and methods

Area description

All three-study areas were located in the Baltic Sea coastal waters of West-Estonian Archipelago (Fig. 1). One of them (Vilsandi) was facing the open Baltic Proper and the other two were located in the more sheltered, inner sea of West-Estonian Archipelago (Väinameri). All three areas represent different complexes of environmental conditions. The Vilsandi area was representing the most 'marine' conditions of the three. The salinity of the surface waters in this area was mostly above 7= psu. It also had the highest water transparency, high variability of substrates and high intensity of wave exposure (Trei, 1973; Mardiste, 1970). The sea area near the islets of Hiiumaa was located in the inner part of Väinameri and was much more sheltered with lower water transparency than in Vilsandi area. The region is located in the dynamic, hydrological front area (Suursaar et al., 1998) separating the two relatively independent water masses of the more saline Kassari Bay from the Suur Strait–Hari Strait waters influenced by the Gulf of Riga. The study area on the southern coast of Vormsi island was the most sheltered and shallow area of the three. The hydrological conditions were influenced by the same frontal area of Hari straight, which results in a relatively unstable environment (salinity changes in the interval of 5.75–7.25 psu) (Suursaar et al., 1998). The investigated areas differed also by the quality of the substrate and the characteristic bathymetry. The area of Vilsandi Island had the largest share of hard substrate and here more observations were made deeper down. All three investigated areas were nature protection areas or national parks (the Vilsandi area belongs to HELCOM BSPA 16 and the Hiiumaa area to HELCOM BSPA 17). Thus, the direct influence from human activities on the biota of the described areas was minimal.

Data collection

Field data were collected from the three areas during two field seasons in 1997 and 1999. The observations were conducted during summer season (August-September) during both years. The material was collected using SCUBA diving. The total number of described locations from each area was: Vilsandi 180, Hiiumaa 98 and Vormsi 120.

In the present scientific literature, two types of data are usually collected for studies of structure of communities. Quantitative data are always best when the purpose is to analyse the community structure by variety of numerical methods. For this, usually the wet weight or dry weight is determined for each benthic macrophyte sample. However, this method has the major disadvantage that determination of biomass is both laborious and time consuming. Another possibility is to use some semi-quantitative measure to characterise both the species composition and the structure of the community. For phytobenthic investigations it is usually species coverage. This measure is sometimes considered to be less accurate describing the structure of the community, but the possibility to collect larger amounts of data seems to compensate for this. During the present investigation coverage estimations were used for the community structure analyses. Data were collected by visual observations by divers along 10–30 m long and 1–2 m wide transects. In addition, at each site geographical position (by GPS), depth, type of the substrate (see Fig. 2 for substrate coding) were recorded. To describe the distribution patterns of the phytobenthic species in each area measures of constancy (C) and dominance (D) were calculated. Constancy was calculated as the share of findings in the total number of observations, dominance as the share of findings where the species was classified dominant or co-dominant in the total number of findings. Species list of macrophytobenthos follows the nomenclature presented in Nielsen et al. (1995).

Data analysis

The classification of the phytobenthic communities was performed using hierarchical, agglomerative and polythetic cluster analyses using untransformed species percent coverage. The data were not transformed

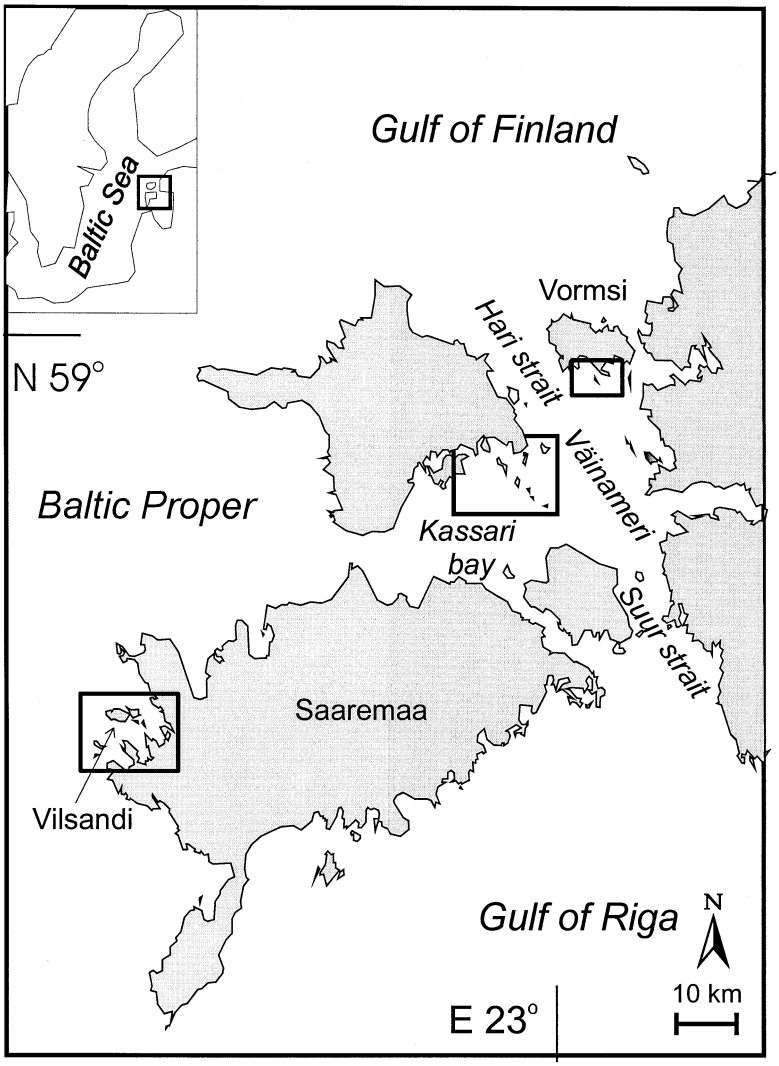

Figure 1. Locations of the three study areas.

in order to give the species with higher coverage values more weight in the classification procedure, taking into account that dominating species have higher importance in formation of the stable community structure. The classification procedure was performed separately for each area. The final classification includes the units in common for several areas.

The linkage method used in the cluster analyses was Wards (1963) method (Ward's method is also known as the 'error sum of squares'). The Euclidean distance was used for distance measure between objects. Clustering was performed by PC-ORD Version 4.01 package (McCune & Mefford, 1999).

Statistical significance of differences along the depth gradient and bottom quality categories was tested by the ANOSIM (Clarke & Green, 1988) technique and the species causing these differences were evaluated using the SIMPER method (Clarke, 1993) included in the PRIMER package.

Results

Phytobenthos species composition

During the study altogether 26 taxa of macroalgae and aquatic higher plants were identified. Among them, six species belonged to the Bangiophyceae, seven species to the Fucophyceae (*Ectocarpus siliculosus* and *Pilayella littoralis* were not distinguished

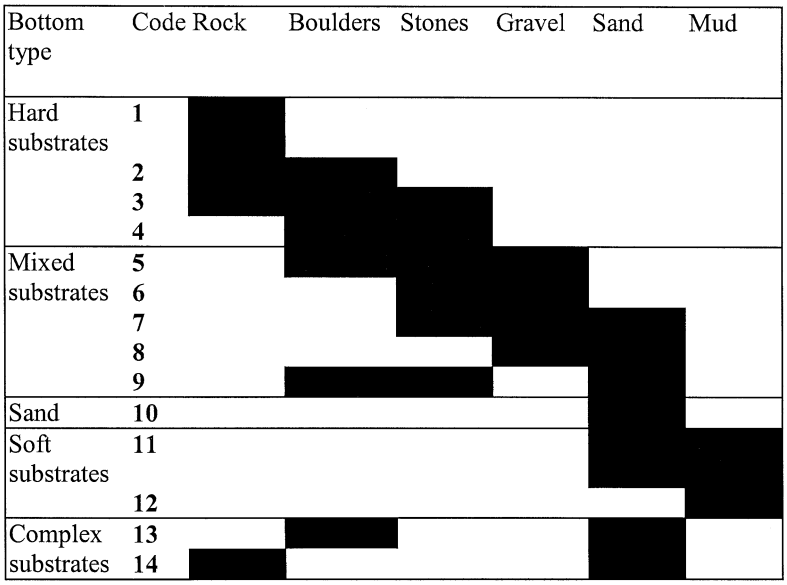

Figure 2. Coding of bottom types (modified from Kautsky, 1989).

from each other due to identification difficulties in field conditions). Of the Charophytes only two taxa were distinguished: *Tolypella nidifica* and *Chara* spp. Chlorophyceae were presented by four species and rooted macrophytes by seven species (Table 1). The species included in the list were only determined by visual observations.

The areas differed in the number of phytobenthic species; the highest species number was observed in Vilsandi (24 species) and the lowest in Hiiumaa region (19 species). The Vilsandi area had more Bangiophyceae and Fucophyceae species compared to the others. In all three investigated areas, one of the most important phytobenthic species in the Baltic Sea, *Fucus vesiculosus,* in general had low constancy. Its highest constancy was in the Vormsi area while the dominancy was low. In the Vormsi area, *Fucus* usually grew as dwarf, unattached individuals and found mainly in communities dominated by phanerogams. Most characteristic in all three study areas was a high dominance and constancy of phanerogams of freshwater origin. The only true 'marine' phanerogam in this region, *Zostera marina*, had low frequency in all areas.

Classification

The first run of the clustering procedure resulted in 17 clusters for the Vilsandi area, 15 clusters for the Hiiumaa area and 15 clusters for the Vormsi area. After examination of the quantitative and qualitative structure of the clusters of the first run, several neighbouring clusters were merged, based mainly on a subjective evaluation of their biotic structure. Perennial species with high coverage values were given priority determining the classification units. After merging, the following numbers of clusters remained: Vilsandi 12, Hiiumaa area 10, and Vormsi area 7. Finally, in total 18 different communities were defined and described. Substrates seemingly lacking vegetation were defined as a separate community characterised by the macrozoobenthos. The parameters of the defined communities are presented in Table 2. The communities were named by the plant species dominating by coverage degree.

Three communities were present in all three investigated areas. They were the sandy substrate without any vegetation (with a frequency of occurrence between 2 to 4% in the areas), the communities of poor vegetation (total coverage less than 5%) occurred with similar frequency of 23–24% in all three areas and the communities dominated by *Zannichellia palustris* with frequencies of 7% in the Vilsandi and Vormsi area and of 11% in the Hiiumaa area. Four communities were present only in the Vilsandi and the Hiiumaa areas. These were the *Ceramium tenuicorne* community (with a frequency of 2 and 3%), the *Cladophora rupestris* community (3% in both areas), the *Potamogeton pectinatus* community (14 and 11%) and the *Potamogeton perfoliatus* community (of 1%

Table 1. List of macrophytobenthos taxa found in three study areas during the field work of 1997 and 1999 with constancy (*C*) and dominancy (*D*) characteristics.

Species	Constancy *C*, %			Dominancy *D*, %		
	Vilsandi	Hiiu-maa	Vormsi	Vilsandi	Hiiu-maa	Vormsi
	1	2	3	4	5	6
BANGIOPHYCEAE						
Coccotylus truncatus (Pall.) Wynne & Heine	1	5	–	0	40	–
Furcellaria lumbricalis (Huds.) Lamour.	3	37	3	32	25	0
Ceramium tenuicorne (Kütz.)Waern	9	14	8	29	14	0
Polysiphonia fucoides (Huds.) Grev.	4	36	1	13	40	0
Rhodochorton purpureum (Lightf.) Rosenv.	1	–	–	0	–	–
Rhodomela confervoides (Huds.) Silva	3	–	1	0	–	0
FUCOPHYCEAE						
Dictyosiphon foeniculaceus (Huds.) Grev.	–	–	2	–	–	0
Stictyosiphon tortilis (Rupr.) Reinke sensu Rosen.	2	–	–	0	–	–
Ectocarpus siliculosus (Dillwyn) Lyngb. / *Pilayella littoralis* (L.) Kjellm.	9	46	14	6	27	6
Elachista fucicola (Velley) Aresch.	1	1	2	0	0	0
Sphacelaria arctica Harv.	2	3	3	0	0	50
Chorda filum (L.) Stackh.	1	–	–	0	–	–
Fucus vesiculosus L.	22	14	78	33	0	16
CHAROPHYCEAE						
Chara spp.	13	12	33	57	8	15
Tolypella nidifica (Müll.) Braun	–	1	1	–	0	0
CHLOROPHYCEAE						
Enteromorpha spp.	20	1	3	33	0	0
Monostroma balticum (Aresch. Ex Wittr.) Wittr.	4	–	3	14	–	0
Cladophora glomerata (L.) Kütz.	22	16	21	51	19	8
Cladophora rupestris (L.) Kütz.	6	8	2	27	38	0
MAGNOLIOPHYTA						
Myriophyllum spicatum L.	10	3	47	17	0	27
Potamogeton pectinatus L.	26	23	76	30	22	32
Potamogeton perfoliatus L.	3	23	18	0	43	5
Ranunculus baudotii Godr.	1	2	23	0	0	7
Ruppia spp.	11	3	32	5	33	24
Zannichellia palustris L.	13	33	14	25	16	47
Zostera marina L.	4	11	3	71	18	33

in the Vilsandi and 11% in the Hiiumaa area). Only one of the communities was present in both the Hiiumaa and the Vormsi areas. This was the *Ruppia* spp. community, which was identified for the Hiiumaa in 1% and in the Vormsi area in 10% of the observations. Two of the defined communities were present in both the Vormsi and Vilsandi areas only. These were the communities of *Fucus vesiculosus* (frequency 12–13%) and *Chara* spp. (with frequency of 7% in both areas). Nine communities were only found in a single area. Among these were communities of *Furcellaria lumbricalis* and unattached *Furcellaria lumbricalis* (occurring with a frequency of 1% and 2% in the Vilsandi area), *Cladophora glomerata–Enteromorpha intestinalis* (occurring with high frequency of 21% on the shallow hard substrates of the

Table 2. List of phytobenthos communities obtained by the classification of the phytobenthos semi-quantitative data from three study areas.

Name of the community	Frequency on investigated area			Depth interval	Substrate	Total coverage	Frequent plant species (Dominating species underlined)
	Vilsandi	Hiiumaa	Vormsi				
1	2	3	4	5	6	7	8
Ceramium tenuicorne	2%	3%	–	0.1–2.2 m	Limestone rock, stones or gravel	70–100%	Ceramium tenuicorne Chorda filum *Enteromorpha* spp. Fucus vesiculosus *E. siliculosus /P. littoralis*
Furcellaria lumbricalis	1%	–	–	4.5–5.8 m	Limestone rock, stones or gravel	75–80%	Furcellaria lumbricalis Polysiphonia fucoides
Furcellaria lumbricalis (unattached)	2%	–	–	1.5–10.0 m	Limestone rock or muddy and sandy gravel	20-100%	Furcellaria lumbricalis Monostroma balticum *Myriophyllum spicatum* *Potamogeton pectinatus*
Furcellaria lumbricalis-Polysiphonia fucoides	–	24%	–	1.0–8.1 m	Different mixed bottoms	30–100%	*Furcellaria lumbricalis* *Polysiphonia fucoides* *E. siliculosus / P. littoralis* *Furcellaria lumbricalis* (unattached) *Coccotylus truncatus* *Potamogeton pectinatus*
Fucus vesiculosus	12%	–	13%	0.1–4.0 m	Mixed bottoms with stones and sand	30–100%	*Fucus vesiculosus* *Ceramium tenuicorne* *E. siliculosus /P. littoralis* *Fucus vesiculosus* (dwarf unattached form) *Potamogeton pectinatus* *Polysiphonia fucoides* *Ruppia* spp. *Cladophora glomerata*
Ectocarpus siliculosus / Pilayella littoralis	–	11%	–	0.6–4.4 m	stony	Not identified	*E. siliculosus / P. littoralis* *Polysiphonia fucoides* *Cladophora glomerata*
Cladophora glomerata	–	5%	–	0.6–3.5 m	Sandy bottoms bottoms with inclusion of gravel and stones	50–80%	*Cladophora glomerata* *E. siliculosus /P. littoralis* *Fucus vesiculosus* *Ceramium tenuicorne*

Continued on p. 157

Table 2. Continued.

Name of the community	Frequency on investigated area Vilsandi	Hiiumaa	Vormsi	Depth interval	Substrate	Total coverage	Frequent plant species (Dominating species underlined)
1	2	3	4	5	6	7	8
Cladophora glomerata-	21%	–	–	0.1–0.3 m	Sand with gravel, limestone rock	50–100%	*Cladophora glomerata* *Enteromorpha* spp. *Ceramium tenuicorne* *Furcellaria lumbricalis* (unattached) *Polysiphonia fucoides* *Potamogeton pectinatus* *Stictyosiphon tortilis* *Zannichellia palustris*
Cladophora rupestris	3%	3%	–	0.1–1.8 m	Sandy gravel or stones	20–60%	*Cladophora rupestris* *Potamogeton pectinatus* *E. siliculosus /P. littoralis*
Chara spp.	7%	–	7%	0.3–2.3 m	Sandy gravel or muddy sand	50–100%	*Chara* spp. *Potamogeton pectinatus* *Myriophyllum spicatum*
Myriophyllum spicatum-Potamogeton pectinatus	–	–	37%	0.3–2.1 m	Muddy sand with some stones	45–100%	*Myriophyllum spicatum* *Potamogeton pectinatus* *Chara spp.* *Fucus micro* *Fucus vesiculosus* *Ranunculus baudotii* *Ruppia* spp. *Zannichellia palustris*
Potamogeton pectinatus	14%	4%	–	0.1–3.0 m	Muddy sand, sometimes with gravel	30–100%	*Potamogeton pectinatus* *Chara* spp. *Ruppia* spp. *Potamogeton perfoliatus* *Myriophyllum spicatum* *Zannichellia palustris* *Zostera marina*
Potamogeton perfoliatus	1%	11%	–	0.8–3.3 m	Sand	30–100%	*Potamogeton perfoliatus* *Potamogeton pectinatus* *Furcellaria lumbricalis* *Myriophyllum spicatum*
Ranunculus baudotii	–	–	37%	0.6 m	Muddy sand	Not identified	*Ranunculus baudotii* *Potamogeton pectinatus*
Ruppia spp.	–	1%	10%	0.2–3.0 m7	Muddy sand, sometimes with stones	50–100%	Ruppia spp. *Potamogeton perfoliatus* *Potamogeton pectinatus* *Chara* spp. *Fucus vesiculosus* *Furcellaria lumbricalis* (unattached) *Myriophyllum spicatum*

Continued on p. 158

Table 2. Continued.

Name of the community	Frequency on investigated area Vilsandi	Hiiumaa	Vormsi	Depth interval	Substrate	Total coverage	Frequent plant species
1	2	3	4	5	6	7	8
Zannichellia palustris	7%	11%	7%	0.1–3.9 m	Muddy sand, sometimes with gravel or stones	20–100% *Fucus vesiculosus*	*Zannichellia palustris* *Ceramium tenuicorne* *Cladophora glomerata* *Potamogeton pectinatus* *Potamogeton perfoliatus* *E. siliculosus /P. littoralis* *Zostera marina* *Chara* spp.
Zostera marina	3%	–	–	3.0–4.0 m	Sand with gravel or mud	70–100%	*Zostera marina* *Furcellaria lumbricalis* (unattached) *Ruppia* spp.
Bottoms with poor vegetation	24%	23%	23%	0.1–4.0 m	Sand with gravel or mud	3–20%	*Cladophora glomerata* *Enteromorpha* spp. *Ruppia* spp. *Fucus vesiculosus* *Potamogeton pectinatus* *Potamogeton perfoliatus* *Chara* spp. *E. siliculosus /P. littoralis* *Furcellaria lumbricalis* *Zannichellia palustris*
Sandy bottoms without vegetation	4%	2%	2%	0.1–4.0 m	Muddy sand or sand	0%	Without vegetation

Vilsandi area), *Zostera marina* community (occurring with frequency of 3% on the sandy substrates of the Vilsandi area). Three communities were attributed only to the Hiiumaa area. These were communities of *Furcellaria lumbricalis–Polysiphonia fucoides* (frequency of 24%), *Ectocarpus siliculosus/Pilayella littoralis* (11%) and *Cladophora glomerata* (5%). In the Vormsi area the communities of *Myriophyllum spicatum–Potamogeton pectinatus* (37%) and *Ranunculus baudotii* (37%) were found.

Environmental forcing

The importance of depth and substrate type for the structure of the phytobenthic communities in the area was tested by comparing depth intervals and substrate types using the ANOSIM test (Fig. 3). In the Vilsandi area a significant difference was obtained for almost all depth intervals up to 5 m depth. Some neighbouring depth intervals were not significantly different but the general result showed a strong correlation to the depth gradient. *Chara* spp., *Potamogeton pectinatus* and *Fucus vesiculosus* mainly caused the differences between communities inhabiting different depth intervals. Other important species causing differences between single depth intervals were *Zostera marina* and *Ruppia* spp.

In most cases, the ANOSIM analysis of substrates showed a significant difference between the sand and/or mud fractions and the substrates lacking sand and/or mud. The main difference was caused by species requiring hard substrate (*Cladophora glomerata*, *Fucus vesiculosus* and also *Furcellaria lumbricalis*). In the Hiiumaa area the results of the ANOSIM test comparing the depth intervals showed similar patterns found in the Vilsandi area. Significant differ-

Table 3. Comparison of communities obtained by the classification of the phytobenthos coverage data from three study areas with the classifications made by other authors.

Name of community	Kukk 1979	Kautsky, Maarel 1990	Trei 1991	Paal 1997	Reitalu 1999 (pers. commun.)	Present investi-gation
Ceramium nodulosum			+			
Ceramium tenuicorne		+			+	+
Ectocarpus siliculosus–Pilayella littoralis	+		+	+		+
Furcellaria lumbricalis	+		+	+		+
F. lumbricalis–Polysiphonia fucoides–Ceramium tenuicorne			+		+	
F. lumbricalis–P. fucoides					+	+
F. lumbricalis–C. nodulosum		+	+			
F. lumbricalis–Coccotylus truncatus			+	+		
F. lumbricalis (unattached)						+
Fucus vesiculosus	+		+	+	+	+
F. vesiculosus–C. tenuicorne		+				
F. vesiculosus–F. lumbricalis	+		+	+		
F. vesiculosus (dwarf form)			+	+		
P. fucoides			+		+	
Pseudolithoderma subextensum	+		+			
Rhodomela confervoides	+		+			
Sphacelaria arctica	+		+	+		
Sph. arctica–filamentous brown algae					+	
Sph. arctica–Polysiphonia fucoides					+	
Cladophora glomerata	+	+			+	+
Cl. glomerata–Enteromorpha spp.						+
Cladophora rupestris					+	+
Enteromorpha intestinalis	+					
E. prolifera	+					
Ulothrix zonata	+					
Chara spp.	+		+	+		+
Myriophyllum spicatum			+	+		
M. spicatum–Potamogeton pectinatus						+
Phragmites australis	+					
Potamogeton filiformis	+		+			
Potamogeton pectinatus	+		+	+		+
P. pectinatus–Zannichellia palustris			+	+		
Potamogeton perfoliatus	+		+			+
Ranunculus baudotii						+
Ruppia spp.	+		+	+		+
Schoenoplectus tabernaemontanii	+					
Zannichellia palustris	+		+			+
Zostera marina	+		+	+		+
Bottoms with poor vegetation		+				+
Sandy bottoms without vegetation						+

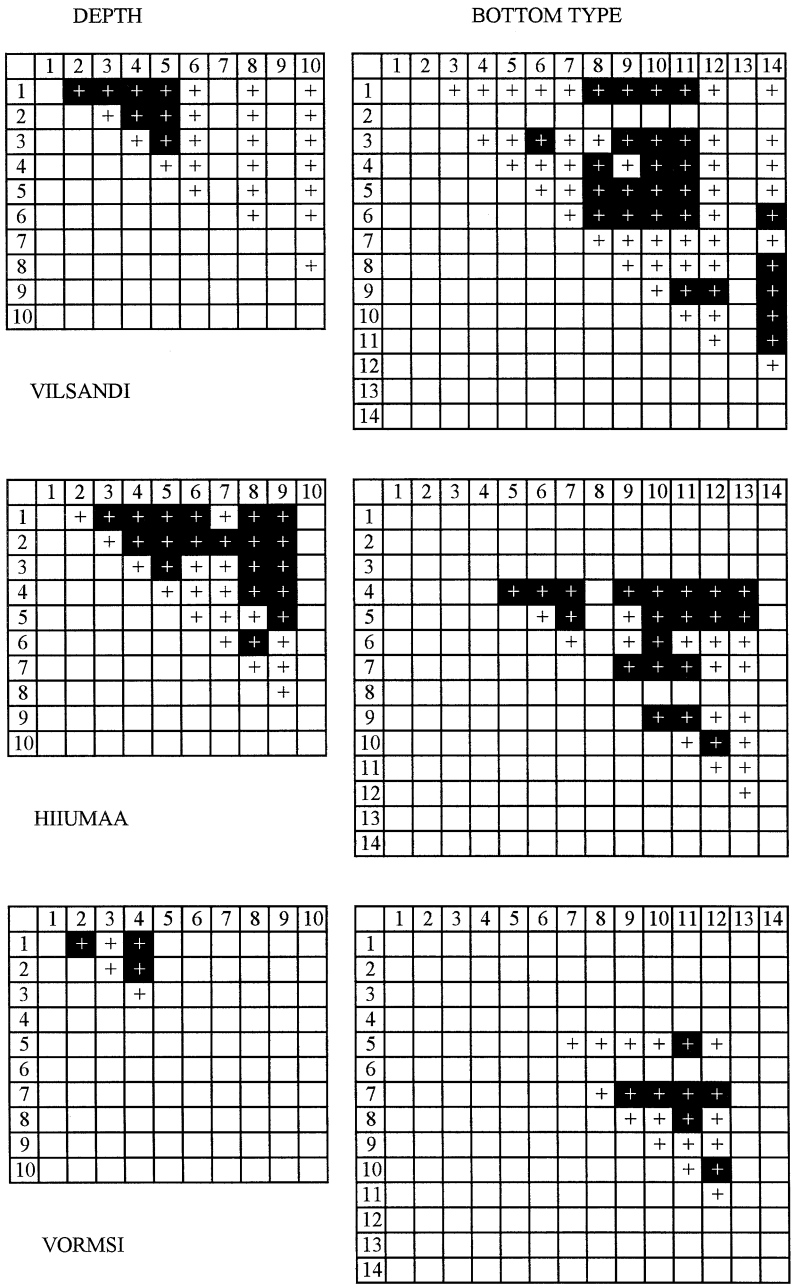

Figure 3. Results of the ANOSIM tests performed on the phytobenthos coverage data of different depth intervals (each interval consisted of 1 m depth) and substrate quality categories (categories based on Fig. 2). (+ represents observed combination, a filled area represents presence of a significant difference between categories, only the upper half of the matrix is shown).

ence was obtained for next neighbour depth intervals while for the neighbouring depth intervals the difference was usually not significant. The difference between the most shallow areas and deeper down was caused by *Pilayella littoralis/Ectocarpus siliculosus*, *Ceramium tenuicorne* and *Potamogeton perfoliatus*, and between shallow and the deepest areas (7–9 m) by *Furcellaria lumbricalis* and *Coccotylus truncatus*. The hard bottoms significantly differed from all other substrates in the area and also bottoms composed of pure sand differed significantly from all the other substrates. The most important species causing the dif-

ferences between communities on different substrates between hard bottoms and others were *Pilayella littoralis/Ectocarpus siliculosus* and *Potamogeton perfoliatus*. The differences between pure sandy bottoms and others were usually caused by different rooted macrophyte species as *Potamogeton perfoliatus*, *Potamogeton pectinatus* and *Zannichellia palustris*.

For the Vormsi region, the results of ANOSIM tests on depth intervals showed a significant difference in community structure only between the shallowest (1–2 m) and the deepest (4 m) intervals. This was mainly due to the distribution of the rooted macrophytes *Potamogeton pectinatus*, *Myriophyllum spicatum* and *Zostera marina*. The results of the ANOSIM test on different substrate types showed only significance between soft and mixed bottoms in general. These differences were mainly caused by *Potamogeton pectinatus* and *Fucus vesiculosus*.

Discussion

Differences in identified species between historic investigations and the present study were apparent. Most of the earlier work included thorough microscopic analysis of the species, therefore is the number of species usually higher in these works compared to the present paper. Nevertheless, several new macroscopic species were found in the areas during the present investigation. For the Vilsandi area the new species were *Potamogeton perfoliatus* and *Ranunculus baudotii*. For the investigated area near the southern coast of Hiiumaa the species *Elachista fucicola* and *Ranunculus baudotii* were not mentioned before. On the other hand, the macroscopic species *Chorda filum* and *Stictyosiphon tortilis* were not found during our investigation. For the Vormsi area, four species were not mentioned in previous publications. These were the red alga *Rhodomela confervoides*, the brown alga *Elachista fucicola* and the phanerogams *Zannichellia palustris* and *Ruppia* spp. The absence of short-lived, filamentous species from the results of previous, historic works could be explained by different sampling technique and determination procedures. The change in occurrence of structuring, perennial species might indicate a trend within the development of the natural phytobenthic communities in the area. A comparison with earlier works indicates the occurrence of new phanerogams in the areas during the last decades. The species composition of the three areas reflects differences in environmental conditions of the areas. The high proportion of red algae in the phytobenthos of Hiiumaa area at shallow depths is well in accordance with high water turbidity in the central part of Väinameri area reported earlier by several authors (Trei, 1970; Suursaar et al., 1998).

The first classification systems of phytobenthic communities for the area were presented by Trei (1973, 1991) and Kukk (1979). Their methods were similar in principles and classification, being based on the empirical interpretation of a large quantitative and semi-quantitative material collected during several decades. Based mainly on the same material, the aquatic part of the 'Classification of Estonian vegetation site types' was recently composed and published (Paal, 1997). Modern numerical classification techniques have not been used before. Recent attempts to apply such methods for Estonian waters were made by Reitalu et al. (2002). An alternative approach of classification was developed by Oulasvirta et al. (2000). They only considered the visual abundance of perennial species. The classification presented in the present paper is similar to the systems published before, confirming most of the classification units defined in previous papers (Table 3). However, the present classification is not fully operative and must be developed further. Improvements should be based on a larger material of quantitative and semi-quantitative data.

According to our study, the influence of depth and substrate was different depending on the area. In the westernmost, most exposed area, the depth gradient showed a strong correlation to the structure of the benthic vegetation while in the central, sheltered area distinct, 1 or 2 m wide depth belts were observed in the phytobenthos. At the same time, we observed lack of a clear vertical zonation in Vilsandi area. Instead, phytobenthos zonation was caused by substrate characteristics in the area.

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Phytobenthos and macrozoobenthos of the Slupsk Bank stony reefs, Baltic Sea

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Key words: Baltic Sea, Slupsk Bank, stony reef, phytobenthos, macrozoobenthos

Abstract

The stony reefs of the Slupsk Bank were studied in July 1999 using hydroacoustic methods, underwater photography and video. Samples of macroalgae and associated fauna were collected by divers using a specially designed sampler. The results of the research indicate that this area is a unique site in the southern Baltic Sea. The high diversity of macroalgae and associated fauna, including the occurrence of three algal species endangered in the Polish coastal zone, is particularly of interest. The Slupsk Bank is also a feeding ground for many wintering birds. For these reasons, it is proposed that the site is designated as an open sea Helsinki Commission Baltic Sea Protected Area (HELCOM BSPA). It is also proposed that it is used as a reference area for ecosystem structure and ecotoxicological studies.

Introduction

This research was undertaken to study the stony reefs of the Slupsk Bank as little is known about this part of the southern Baltic Sea bottom. The principal aim of this work was to determine if it is justified to establish an offshore Baltic Sea Protected Area (Andrulewicz et al., 1996; Andrulewicz & Wielgat, 1999), and, if so, to propose its boundaries and make recommendations for a monitoring scheme. An additional aim was to test modified gear for sampling macroalgae and associated fauna.

Our knowledge of stony Baltic reefs is very limited because they are too shallow to be navigated by larger research vessels and cannot be sampled using traditional sampling gears. Quantitative sampling on shallow stony bottoms requires scuba diving (Kautsky, 1993; Olenin, 1995), but the technique is expensive, difficult and needs reasonably calm weather conditions. As a result, the deep basins of the Baltic Sea are investigated more frequently than are its shallow areas. This is why very little is known about the Slupsk Bank stony reefs located in the central part of the southern Baltic Sea in the Polish exclusive economic zone approximately 25 km north of the Polish coast (Andrulewicz & Wielgat, 1999; Fig. 1).

The area was sampled for the first time in the early fifties by Demel & Mankowski (1951) followed by Demel & Mulicki (1954) and then in the late seventies by Warzocha (1980). However, the soft bottom samplers utilised then were inappropriate for sampling stony bottoms. Okolotowicz (1991) conducted qualitative studies of the epiphytic flora and fauna on stones collected by divers. Therefore, the current study is the first attempt to assess quantitatively and qualitatively epiphytic flora and associated fauna in the same underwater sampling event.

A hard, stony area occurs at depths between 8 and 20 m in the northwestern part of the bank (Fig. 2). The remaining 80% of the Slupsk Bank is covered by almost flat plains of sand and gravel deposits (Maslowska, 1993). The water masses above the Slupsk Bank reefs are polymictic and are therefore characterized by isothermic and isohaline hydrological conditions (IMWM, 1987–2000). The bank is

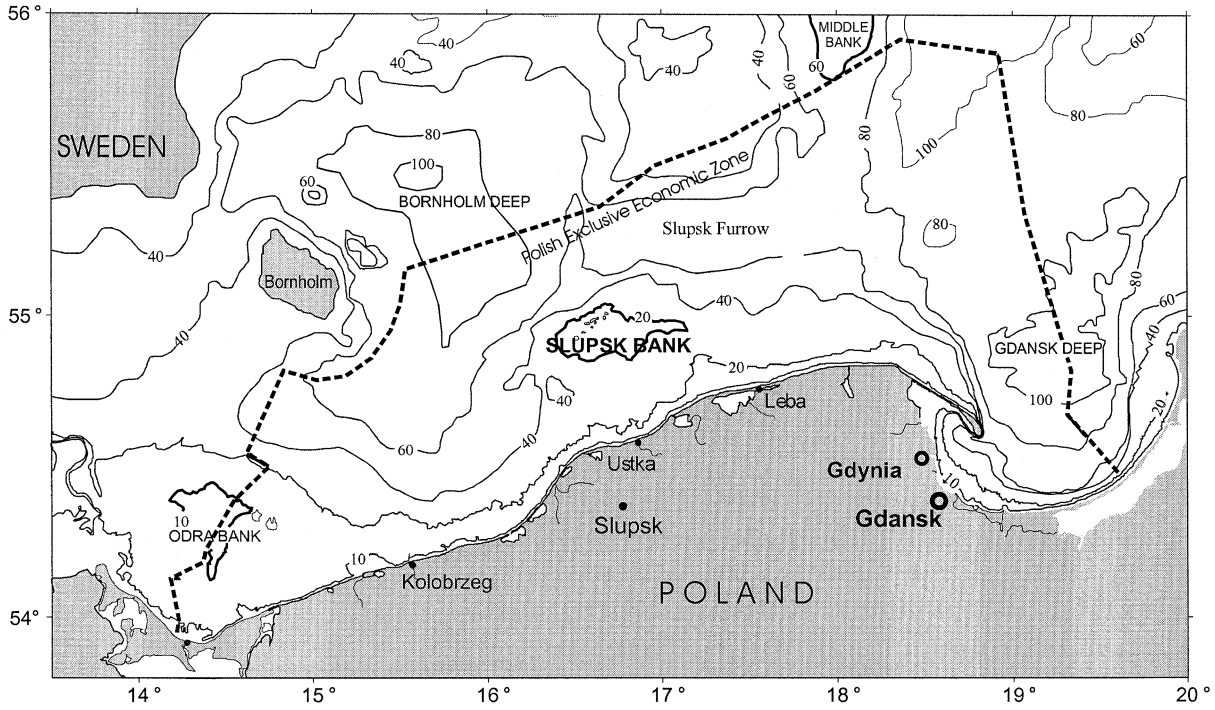

Figure 1. Geographic location of the Slupsk Bank within the Polish exclusive economic zone.

exposed to wavy motion, which results in a low level of organic matter and good oxygen conditions (Gajewski & Uscinowicz, 1993, Szczepanska & Uscinowicz, 1994).

Due to its offshore location, pollution of land origin does not directly influence the Slupsk Bank (Andrulewicz & Wielgat, 1999) although large-scale anthropogenic effects such as eutrophication and contamination must impact the Slupsk Bank biocenosis to some degree. The Secchi disk transparency varies from 6 to 12 m during the summer (Andrulewicz & Wielgat, 1999), which means that light reaches a significant portion of the bank's bottom. Fishery on the stony reefs is limited to passive gill nets because of obstructions to bottom trawling (Kruk-Dowgiallo, 2001; J. Netzel, pers. commun.). Therefore, the Slupsk Bank reefs can be regarded as an undisturbed bottom habitat.

Materials and methods

On-site studies of the stony parts of the Slupsk Bank were performed in July 1999 on a selected study polygon in the northwestern part of the bank (Fig. 2). A DGPS positioning system, acoustic profiling with DGPS and a computer, closed-circuit TV, scuba diving for observation and sampling, underwater photography and video were used during the study. The shallowest areas of the bank were selected using navigational charts before the study commenced. The acoustic profiler with DGPS and the computer were used to record the profile parameters and to identify the stony sites (Fig. 3). Surprisingly, the information recorded en route did not always correspond to that on the navigational charts.

Water transparency was measured daily using a Secchi disk. Preliminary screening at selected stony sites was conducted at low speeds using the acoustic recorder and closed-circuit TV. Following this, it was decided whether or not to use scuba divers for sampling (Kautsky, 1993; HELCOM, 1999). Underwater photos and video were also made to document the area and sampling effectiveness (Andrulewicz, 2000). Altogether, 20 stations were documented on video by scuba divers and 36 samples of macroalgae and associated fauna were collected by the divers at two or three depths (8.5 m, 12–13 m, 16.5–18 m).

The macroalgae and associated fauna were sampled using a modified Kautsky sampler for hard bottoms with a catch area of 400 cm^2 (Kautsky, 1989, 1993, 1995; Fig. 4). The samples were brought aboard the vessel and divided into two sub-samples of mac-

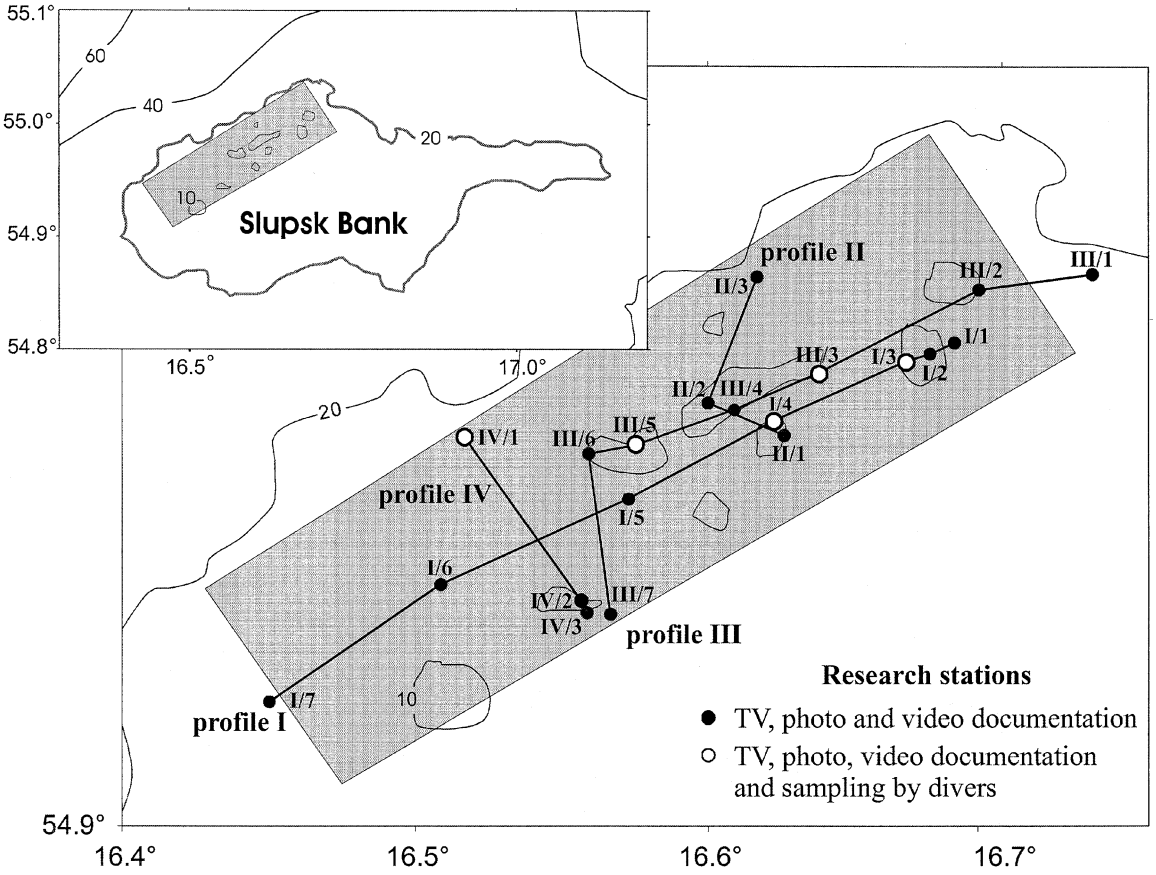

Figure 2. Study polygon on stony areas in the eastern part of the Slupsk Bank.

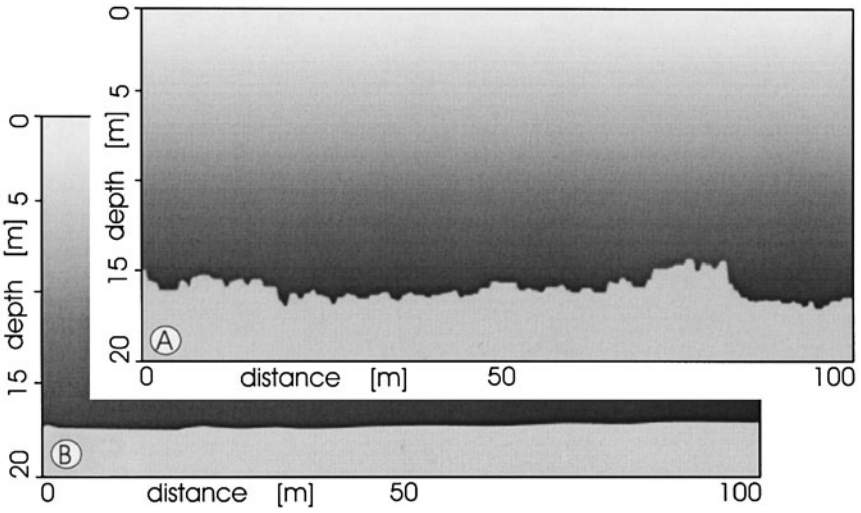

Figure 3. Examples of acoustic records of sampling sites: (a) stony bottom, (b) sandy bottom.

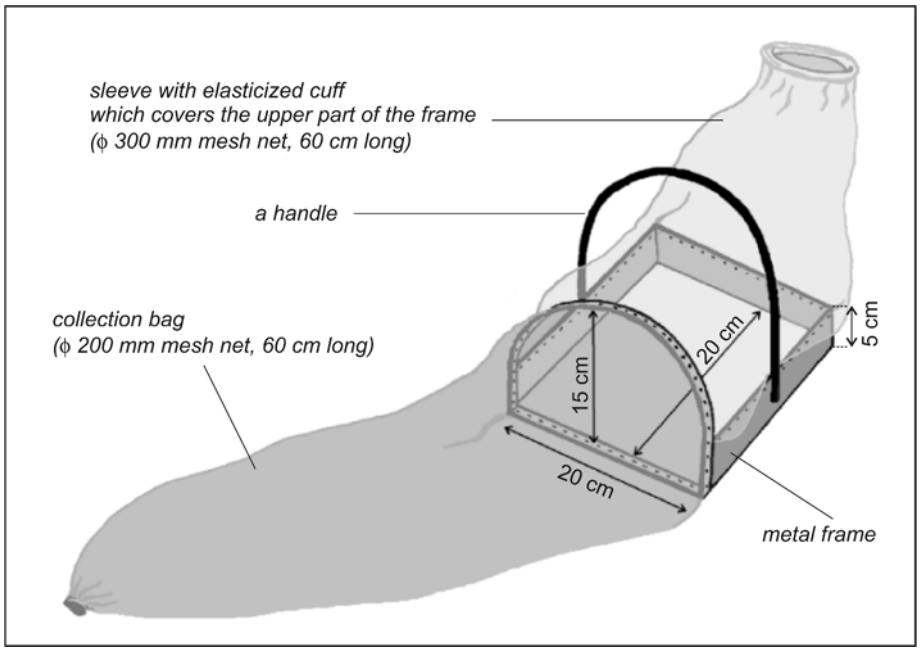

Figure 4. Modified Kautsky sampler for quantitative sampling of underwater flora and associated fauna.

roalgae and associated fauna. The algae were frozen and the fauna was preserved in a 4% formalin solution (Dybern et al., 1976; HELCOM, 1999). After the species had been identified, the macroalgae were collected and stored in a herbarium. Sorting, identifying the species and determining the biomass of the bottom macrozoobenthos was done in accordance with HELCOM guidelines (HELCOM, 1988). Nematoda, Nemertinea, Oligochaeta and Insecta larvae were not identified to the species level.

Results

Secchi disk transparency

Secchi disc transparency varied from 8.5 m at the beginning of the study to 6 m at the end. The decreasing transparency was caused by a progressing algal bloom.

Bottom morphology

The stones observed were classified as very large (more than 1 m in diameter), large (0.5–1 m), and small (0.25–0.5 m). Small stones were the most common and often they occurred together with large and very large ones. The distribution of the stones on the Slupsk Bank was generally patchy and formed hills.

The shallowest parts of the Slupsk Bank (generally about 8–12 m of depth) were dominated by vegetation. At deeper levels, the stones were covered with blue mussels (*Mytilus edulis trossulus*) and barnacles (*Balanus improvisus*). The stony parts of the Slupsk Bank can be described as a series of underwater islands which provide habitats that are significantly different from their sandy surroundings. Ripple marks in the sand indicate that the bottom at a depth of 20 m was still highly dynamic.

Bottom vegetation

In total, 16 species of macroalgae were identified (Table 1) as follows: Fucophyceae (brown algae: 4 species), Bangiophyceae (red algae: 10 species), Chlorophyceae (green algae: 2 species). One macroalgal species was not identified as it was not sampled (due to technical reasons) and only recorded on video (Andrulewicz, 2000). Three of the species growing at Slupsk Bank, *Delesseria sanguinea*, *Furcellaria lumbricalis* and *Coccotylus truncatus*, co-occur only in this location in the Polish exclusive economic zone.

The most diverse macroalga community was observed at depths from 11.5 m to 13.0 m. The highest macroalgal biomass, from 39 g m^{-2} to 90 g m^{-2} dry weight, was also noted at this depth range and consisted mainly of *Furcellaria lumbricalis.* Red algae

Table 1. List and depth distribution of macroalgal species (taxonomy according to Nielsen et al., 1995).

Taxon	Depth range (m)
Chlorophyceae	
Cladophora glomerata (L.) Kütz.	8.5
Ulothrix flacca (Dillwyn) Thur. in LeJol.	8.5
Fucophyceae	
Pilayella littoralis (L.) Kjellm.	8.5–16.5
Ectocarpus siliculosus (Dillwyn) Lyngb.	8.5–16.5
Sphacelaria cirrosa (Roth) C. Agardh.	13.0–15.0
Dictyosiphon foeniculaceus (Huds.) Grev.	10.0–16.5
Bangiophyceae	
Ceramium diaphanum (Lightf.) Roth	8.5–16.5
Ceramium tenuicorne (Kütz.) Wærn	8.5–13.0
Ceramium nodulosum (Lightf.) Ducluz.	10.0–16.5
Delesseria sanguinea (Huds.) J. V. Lamour.	11.5–15.0
Furcellaria lumbricalis (Huds.) J. V. Lamour.	10.0–16.5
Coccotylus truncatus (Pall.) M. J. Wynne & J. M. Heine	10.0–16.5
Rhodomela confervoides (Huds.) P. C. Silva	10.0–16.5
Polysiphonia fucoides (Huds.) Grev.	10.0–16.5
Ahnfeltia plicata (Huds.) Fr.	13.0
Hildenbrandia rubra (Sommerf.) Menegh.	13.0–15.0
Unidentified alga – recorded on underwater video	13.0

dominated not only in the number of species but also in biomass which constituted 68% of the total. The biomass share of *Furcellaria lumbricalis* was 27% while that of two filamentous brown algae species, *Pilayella littoralis* and *Ectocarpus siliculosus*, together constituted 33%. Generally, the same type of vegetation was observed at the same depths throughout the investigated area. Some larger seaweeds (e.g. *Furcellaria lumbricalis*) as well as some fish and crustaceans were more frequently observed in sheltered places between the largest stones.

Filamentous brown algae were observed attached to the stones in the shallower parts of the bottom, but in the deeper parts they were free-floating. Following two days of rough weather during the study period, large amounts of filamentous algae which had become detached from the hard bottom were observed loose-lying in the deeper parts of the bank. It is likely that these algae are transported to the open Baltic Sea where they form mats on the bottom. The presence of filamentous brown algae in such high quantities as we observed in the present study is a disturbing phenomenon (Kangas et al., 1982; Kruk-Dowgiallo, 1996; HELCOM, 1996) and in earlier studies they have not been found in such massive quantities (Okolotowicz, 1991).

Fauna associated with macroalgae

Twenty-eight faunal taxa, including ten crustacean species, were identified in the macroalgal samples from the stony bottom (Table 2). The blue mussel (*Mytilus edulis trossulus*) biomass was particularly high (3182 $\pm$ 1399 g m^{-2} wet weight). According to earlier studies by Warzocha (1980, 1995) and Osowiecki (1999), the neighbouring sandy and gravel bottom hosted approximately 21 taxa including 11 crustacean species. It is worth noting that we found three different species of *Gammaridae*, the rare crustacean *Calliopius laeviusculus* and a great number of Turbellaria. Fragile, bushy colonies of Hydrozoa were observed only in their natural environment because of the damage which would have been inflicted during sampling. The highest number of taxa, 17–19, was observed at the deeper stony stations at 15-16 m of depth.

Table 2. List of macrofaunal species on the Slupsk Bank reefs. Hydrozoa were observed underwater and recorded on video. n.i. = not identified to the species level.

Taxonomic group	Species
Turbellaria	*Dendrocoelum lacteum* (Müller)
	Planaria torva (Müller)
Nematoda	n.i.
Nemertinea	n.i.
Polychaeta	*Harmothoe sarsi* (Malmgren)
	Hediste diversicolor (Müller)
	Pygospio elegans (Claparede)
	Fabricia sabella (Ehrenberg)
Oligochaeta	n.i.
Hirudinea	*Piscicola geometra* (Linnaeus)
Arachnoidea	*Lochmanella falcata* (Hodge)
Crustacea	*Balanus improvisus* (Darwin)
	Idotea balthica (Pallas)
	Saduria entomon (Linnaeus)
	Jaera syei (Bocquet)
	Calliopius laeviusculus (Kröyer)
	Gammarus zaddachi (Sexton)
	Gammarus salinus (Spooner)
	Gammarus oceanicus (Segestrale)
	Mysis mixta (Lilljeborg)
	Praunus inermis (Rathke)
Insecta (larvae)	n.i.
Gastropoda	*Hydrobia ulvae* (Pennant)
	Theodoxus fluviatilis (Linnaeus)
	Embletonia pallida (Alder, Hancock)
Bivalvia	*Mytilus edulis trossulus* (Gould)
	Macoma balthica (Linnaeus)
Bryozoa	*Electra crustulenta* (Pallas)

Discussion

Currently, the main threat to the Slupsk Bank reef vegetation is the eutrophication of the water masses of the Baltic Sea proper (IMWM, 1987–2000) which hinders light penetration to the deeper water layers. Further increases in eutrophication may promote the growth of more filamentous algae which would change the biocenotic structure of the macroalgae. Other threats are related to the continuing exploitation of living resources, which is not intense at present (J. Netzel, pers. comun.). However, the exploitation of mineral resources might intensify in the future. The large gravel deposits at depths between 16 and 20 m in other parts of the Slupsk Bank cover about 31 km^2 (Kramarska, 1989; Maslowska, 1993).

The ongoing exploitation of sand and gravel deposits in adjacent areas as well as plans to construct wind energy plants on the eastern part of the Slupsk Bank are potential threats to the benthic community on the Slupsk Bank reefs. Adverse effects may be related to changes in bottom morphology, as well as by suspended matter plumes.

Uneven, patchy bottom coverage by *Mytilus edulis trossulus*, particularly in the shallow parts of the bottom, was observed by the divers. This probably resulted from ducks and/or flatfish feeding on the reefs; however, no quantitative measurements of this phenomenon were taken. Another interesting observation made by the divers was the higher water transparency close to the mussel beds. It is possible that intensive filtration by *Mytilus edulis trossulus* can diminish the amount of suspended matter in the seawater to such an extent that it improves water transparency.

Various fish were observed during underwater sampling and included sand gobies *Pomatoshistus minutus*, flatfish (mainly turbot *Scopthalmus maximus*), eelpout *Zoarces viviparus* and cod *Gadus morhua*. Stony reefs are known to provide shelter and be suitable spawning and feeding grounds for different fish species (Ojaveer & Kaleis, 1974; Jansson & Kautsky, 1977; Okolotowicz, 1991).

The bottom primary productivity consists mainly of macroalgae, which are useful both for habitat creation and as an element of primary production in the food chain. The productivity of banks originates from physical parameters like the upwelling phenomena on the slopes of banks and very intensive turbulence and water layer mixing that stimulate a rich and diverse productivity (Ojaveer & Kaleis, 1974).

Stony bottoms offer suitable substrates for a range of macroalgae and fauna species, as well as shelter and feeding grounds for some larger animals. Stony beds provide microhabitats which attract species such as small crustaceans and fish for feeding, reproduction and shelter. Some red algal species (e.g. *Furcellaria lumbricalis*) are primarily found in unexposed areas which are less affected by wavy motion and currents. Thus, the diversity of species in the shallow stony areas of the bottom is higher.

The vertical distribution of macroalgae depends mainly on the availability of light. Vegetation in the study area was observed at depths to 20 m (Andrulewicz, 2000), while on the hard bottom of the Polish coast it only occurs at depths of up to 8 m (Plinski, 1990; Kruk-Dowgiallo, 1998; Osowiecki & Zmudzinski, 2000). In general, the depth range of

macrophytes in the Baltic Sea is about 15 m (Bäck et al., in press). The good light penetration at the Slupsk Bank supports vegetation at much deeper levels than in the coastal areas. This is evidenced by the presence of *Furcellaria lumbricalis* at a depth of 16.5 m. The depth distribution of the maximum biomass of *Furcellaria lumbricalis* (11.5–13.5 m) indicates that it may have been replaced by opportunistic filamentous brown algae in shallower areas.

The Slupsk Bank reefs are possibly a spawning ground for herring and other commercial species such as flatfish and for non-commercial fish such as eelpout and gobidae (J. Netzel, pers. commun.). However, the ichthyofauna of the Slupsk Bank is not well documented. This area is not subjected to intensive fishing, the catches made here comprise only 0.07% of the total catch in the Polish exclusive economic zone and the fishing gear used is usually restricted to passive gill nets and hooks.

The Slupsk Bank is also known to be an important marine area for wintering birds, particularly the long-tailed duck *Clangula hyemalis* (up to 50 000 specimens during winter) (Gorski & Strawinski, 1986). About 10% of the Baltic population of black guillemot *Cepphus grylle* (2000 specimens) has been observed here (Durinck et al., 1994; Meissner, 1994). Feeding by both birds and flatfish could have been responsible for the uneven distribution of blue mussel *Mytilus edulis trossulus* aggregations which were observed by the divers.

According to HELCOM (1998), the Slupsk Bank reefs can be categorized as either a stony bottom in the sub-littoral photic zone (HELCOM code 2.2.2) or as a sub-littoral level stony bottom dominated by macrophyte vegetation (code 2.2.2.2). Another option would be to categorize them as sublittoral mussel beds of the photic zone (code 2.9.2). According to EUNIS (EEA, 1999), the stony bottom parts of the Slupsk Bank can be classified as hydrolittoral mussel beds dominated by macrophyte vegetation (code A1.4/H-02.09.03.02).

Conclusions

Stony reefs are important components of the Baltic Sea ecosystem. They are very productive, diverse habitats and are also important sites for fish and wintering birds. They can be regarded as 'islands of diversity' within the dynamic sandy bottom that surrounds them. At present, they are under strong anthropogenic pressure from sand and gravel extraction activities and plans for the construction of electric windmill farms.

The Slupsk Bank reefs are unique within the generally sandy Polish exclusive economic zone. They appear to provide a suitable substratum for macroalgae and mussels, favourable grounds for invertebrates, food resources, spawning grounds and shelter for juvenile fish and a food resource for wintering birds. It is also a unique habitat for some species of red algae, i.e. *Delesseria sanguinea, Furcellaria lumbricalis* and *Coccotylus truncatus*, which are endangered in the Polish exclusive economic zone.

It is proposed that the Slupsk Bank reefs area is declared a HELCOM BSPA. The size of the site is approximately 110 km^2 with depths ranging from 8.5 to 20 m, and it is located within the following coordinates: 54° 56′ 54″ N, 16° 25′ 36″ E; 55° 02′ 18″ N, 16° 40′ 42″ E; 54° 54′ 24″ N, 16° 28′ 30″ E; 54° 39′ 30″ N, 16° 43′ 30″ E. It is also proposed to establish monitoring sites for macrophytes and associated fauna for the HELCOM COMBINE program as well as for marine biodiversity studies (Bäck et al., in press; Bonsdorff & Blomqvist, 1992). The recommended monitoring stations are those with the highest abundance and biomass located at 54° 59′ 21″ N, 16° 38′ 14″ E and 54° 58′ 30″ N, 16° 34′ 30″ E (Fig. 2 – Stations III/3 and III/5). The modified Kautsky sampler used during this study is recommended for sampling macroalgae and associated fauna for future phytobenthos monitoring.

It is also proposed that the Slupsk Bank reefs be considered as one of the EU Water Framework Directive (1999) reference areas for hard bottoms, and in particular for studies of macroalgae and macrozoobenthos, biological diversity as well as chemical contamination and ecotoxicology.

Acknowledgements

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Climatic and hydrological controls over the zoobenthos in a southern Baltic coastal lagoon

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Key words: Baltic Sea, estuary, meiobenthos, macrobenthos, climate, hydrology

Abstract

In a four-year-long (1985–1988) study of macro- and meiobenthos of the Szczecin Lagoon, a eutrophic and polluted coastal water body directly connected with the southern Baltic Sea, peculiar interannual dynamics of variables related to abundance was observed. The interannual component of variability was stronger than fine-scale seasonal dynamics. The interannual variations followed a trend whereby abundance of meio- and macrobenthos as well as biomass of the latter was high in 1985, dropped in 1986 and 1987, and rose again in 1988. As the trend could not be explained by local environmental data, explanation was sought by invoking wider-scale climatic and hydrological processes. Data for severity of winters preceding each year of study as well as trends in atmospheric circulation patterns, wind regimes, river runoff characteristics, and near-bottom conductance (used as a proxy for seawater intrusions into the lagoon) were compared to seasonal anomalies in meiobenthic abundance as well as in macrobenthic abundance and biomass. The seasonal mean meiobenthic log abundance anomalies produced no significant correlation with any of the variables tested. In contrast, the seasonal mean log macrobenthos abundance and biomass anomalies were significantly negatively correlated with winter severity proxies (mean winter temperature and cold sum) and flow rate determined with time lags generally longer than one month. The results provide indication of differences in responses of meio- and macrobenthic communities to climatic (winter severity) and hydrological changes, the long-term effects being visible primarily within the macrobenthos. However, establishing sound causal relationships requires longer time-series of data.

Introduction

Recent global-scale climatic change has prompted research into the ecological effects of climatic forcing (Hurrell, 1995). Such research has resulted in a realisation that large-scale phenomena such as El Niño Southern Oscillation (ENSO) or the North Atlantic Oscillation (NAO) affect not only local climate and weather in different parts of the Earth (Dickson, 1997), but may also influence long-term patterns of variability in the terrestrial and aquatic ecosystems (Post et al., 1999; Hänninen et al., 2000; Straile & Adrian, 2000; Neira et al., 2001; Ottersen et al., 2001).

Studies on time series of marine communities in the North and Baltic Seas have demonstrated that interannual or periodic variations in water temperature, salinity, dynamics of water masses, water column productivity, zooplankton, and macrobenthos are coupled to changing climate (Kröncke et al., 1998; Tunberg & Nelson, 1998; Beare & McKenzie, 1999; Hänninen et al., 2000; Dippner et al., 2001).

To investigate cause and effect relationships, long time series of data are needed, preferably spanning 20 years or more, and studies are likely to be conclusive whenever such data series exist (e.g., Gerten & Adrian, 2000, 2001; Dippner et al., 2001). There are, however, situations when long time series are lacking, yet there is nevertheless strong evidence of year-to-year variability, the pattern of which cannot be explained

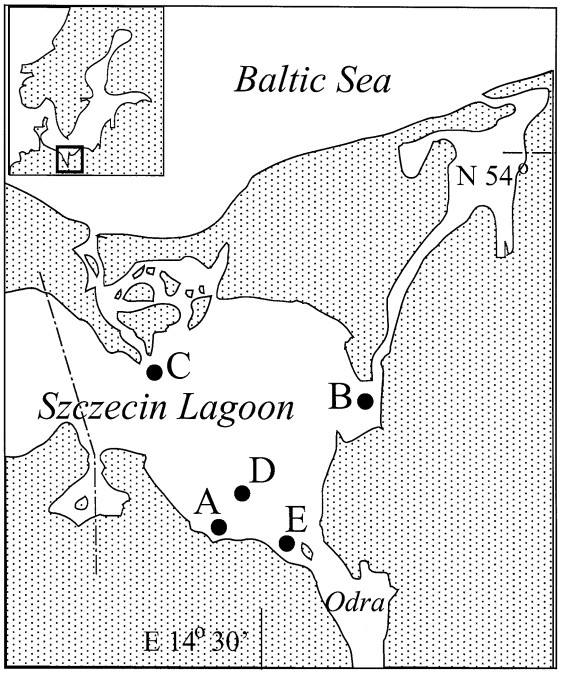

Figure 1. Szczecin Lagoon, with location of stations visited in 1985–1988 (A–E).

by locally collected abiotic data. To explain such variations, it is reasonable to investigate effects of more distant parameters.

In 1985–1988, the meio- and macrobenthos of the Polish part of the Szczecin Lagoon (Oderhaff), a eutrophic and polluted (Lampe, 1993; Protasowicki et al., 1993; Osadczuk et al., 1996) Southern Baltic-connected coastal water body (Fig. 1) was studied (Radziejewska & Drzycimski, 1988, 1990; Masłowski, 1992, 1993). The results showed a peculiar variability in the meio- and macrobenthos: regardless of spatial and seasonal changes within each year, the year-to-year variations involved high abundance and biomass values in the first year of study (1985), followed by much reduced values in the second, and particularly in the third year (1986 and 1987, respectively), and a seemingly slow pick-up in the fourth and final year (1988).

The recent awareness of and emphasis on climate- and hydrology-induced variability in biotic components of numerous aquatic ecosystems (e.g., Kröncke et al., 1998; Gerten & Adrian, 2000, 2001; Hagberg & Tunberg, 2000; Straile & Adrian, 2000) has led us to reassess the 1985–1988 Szczecin Lagoon benthic data in light of climatic and hydrological changes over that period. In this study we therefore try to find out if benthic communities, while showing spatial and seasonal variability, also respond to modulation by interannual variability of climatic and hydrological processes. We also address the question if a four-year-long period of observations is sufficient to justify linking the otherwise unexplicable interannual variability in the benthos to changes in the climatic and hydrological regimes of the water body under study.

Study area

The Szczecin Lagoon (Oderhaff; Fig. 1) is a 687 km^2 coastal reservoir, forming a link in the river Odra (Oder) estuarine system and divided by the state border between Poland and Germany. The lagoon connects with the Pomeranian Bay in the southern Baltic Sea via 3 outlets. The water budget of the lagoon is dominated (98%) by the Odra input, the river discharging an estimated 15–17 km^3 of water per year (Robakiewicz, 1993; Mutko et al., 1994). While the lagoon's water is predominantly fresh, it also receives salt water intrusions from the Baltic Sea (Mutko et al., 1994).

The lagoon is shallow, with depths generally not exceeding 7 m, except for the sub-meridional shipping channel, dredged and maintained for the purpose of making it possible for ocean-going cargo vessels to call at the port of Szczecin. As a result of the complexity of the lagoon's hydrography (Robakiewicz, 1993), the bottom is characterised by the presence of extensive depositional and some erosional areas. The first are covered by a thick layer of organic mud, while the sediment of the other consists of sand, frequently mixed with abundant subfossil mollusc shell remains and ostracod valves and covered by a thin layer of a mobile organic-rich fluffy material (Osadczuk et al., 1996; B. Wawrzyniak-Wydrowska, pers. obs.; T. Radziejewska, pers. obs.).

At present, the lagoon is highly eutrophic, the eutrophication being manifested as high nutrient levels, high phytoplankton standing stocks (water chlorophyll *a* concentrations reaching 220 mg m^{-3}; Mutko et al., 1994) and annual cyanobacterial blooms (Mutko et al., *op. cit.*). The lagoon is also polluted as a result of receiving, for a number of years, water from the Odra (and that of a number of small tributaries), laden with industrial, municipal, and agricultural waste (Protasowicki et al., 1993; Mutko et al., 1994.; Osadczuk et al., 1996).

Table 1. Ranges of near-bottom water characteristics measured during the sampling occasions at the Szczecin Lagoon stations in 1985–1988.

Sampling date	Temperature (°C)	Dissolved oxygen content (mg dm^{-3})	pH	Chlorophyll *a* content (mg m^{-3})
17 Apr. 1985	8.8–9.5	10.2–11.0	7.7–8.1	17.2–60.4
10 June 1985	15.0–18.0	4.8–8.0	8.2–9.9	32.7–93.9
30 Oct. 1985	13.2–14.0	6.4–10.2	7.7–8.6	22.4–33.6
12 Nov. 1985	4.0–4.2	9.3–9.6	7.7–8.2	no data
17 Apr. 1986	6.0–15.0	7.2–13.1	8.0–9.1	25.1–96.7
27 June 1986	20.2–21.9	6.0–10.0	7.8–9.0	31.4–71.1
5 Sep. 1986	10.2–10.3	9.9–11.6	8.3–9.2	72.4–142.8
5 Nov. 1986	6.6–7.2	10.5–11.6	8.3–8.5	42.7–99.3
21 May 1987	11.9–13.2	12.1–14.7	8.2–8.8	13.2–43.3
7 July 1987	19.3–22.4	7.5–12.1	8.1–8.9	22.5–80.9
10 Sep. 1987	15.0–16.0	8.6–15.0	8.0–8.8	30.0–78.9
5 Nov. 1987	5.5–7.0	9.4–13.7	8.1–9.0	7.5–10.6
12 May 1988	14.0–16.5	8.5–11.1	8.2–9.0	18.9–33.7
27 June 1988	18.5–19.0	4.1–8.6	7.9–8.5	19.3–56.9
1 Sep. 1988	18.5–19.0	6.0–10.7	8.6–9.3	25.3–41.8
8 Nov. 1988	4.5–5.5	9.4–10.0	8.0–8.4	8.1–21.7

The study described in this paper spans the period of 1985–1988 and involves the lagoon's meio- and macrobenthos collected from 5 stations (Fig. 1) four times each year in spring (April or May), summer (June or July), late summer (September), and autumn (November). No samples were collected in winter. Table 1 lists dates of sampling and summarises the ranges of environmental data collected concurrently with biological samples.

Materials and methods

Abiotic data

The range of climatic and hydrological variables and parameters utilised in this study include those expressing the severity of winters preceding each sampling year, the type of atmospheric circulation, wind direction and speed, flow rate, water level, and near-bottom water conductance. The severity of the winter preceding each year of study was described by using the mean winter (December–March) temperatures and cold sums (i.e. sums of daily mean sub-zero temperatures over December–March) for the Szczecin Lagoon area. The data were provided by Mr. S. Matalewski, formerly of the Maritime Academy in Szczecin, Poland.

Atmospheric circulation 'type' on each sampling date and over various periods preceding it (1, 3, and 6 months) was determined based on the atmospheric circulation classification system for Central Europe, developed by Lityński (1969). The system identifies, based on zonal and parallel circulation indices and atmospheric pressure over Poland, 27 circulation types. In this study, the averaged circulation types for 1984–1988 listed in the catalogue of atmospheric types (Stępniewska-Podrażka, 1991) were grouped into the 8 major circulation directions (N, NE, E, SE, S, SW, W, NW), the directions being coded (1–8, respectively, and 0 to denote the non-advective situation) for the purpose of numerical treatment of data. The prevailing (most frequent) circulation types were identified for each month of 1984–1985 and for 1, 3 and 6 months preceding each sampling event.

The prevailing (most frequent) wind direction and average wind speed was determined for each month of the period of study as well as for the periods preceding each sampling event, based on records made at 12.00 h GMT at Trzebież on the Szczecin Lagoon, available from the archives of the Institute of Meteorology and Water Management in Warsaw, Poland. For the purpose of numerical treatment of data, wind directions were coded identically as the circulation types.

The magnitude of river runoff into the lagoon was represented in the analyses by a proxy in the form of monthly average flow rates of the Odra, as determined from measurements taken during 1984–1988 at a gauge post located just upstream of the city of Szczecin. The mean monthly water level in Szczecin was another proxy, reflecting a net result of river runoff and the lagoon water being pushed upstream by wind and/or by salt water ingresses from the Baltic Sea (the so-called 'return flows'). Both sets of values were calculated from data extracted from the archives of the Institute of Meteorology and Water Management in Warsaw, Poland. Another representation of the strength of salt water intrusions into the lagoon was the near-bottom water conductance, measured at each sampling event.

Biotic data

The data on mean abundances of the meiobenthos as well as mean abundances and biomass of the macrobenthos at each station on each sampling event, extracted from Radziejewska & Drzycimski (1988, 1990) and Masłowski (1992, 1993), were used to calculate log abundance (meio- and macrobenthos) and log biomass (macrobenthos) anomalies (i.e., deviation from the seasonal mean over the entire period of study) and the relevant seasonal mean log abundance and biomass anomalies. The macrobenthos abundances and biomass values used did not include the contributions by *Pelmatohydra oligactis*, *Dreissena polymorpha,* and *Anodonta anatina*. These species appeared sporadically in the samples, but whenever they did, they disproportionately affected the abundance and/or biomass (Masłowski, 1992).

Numerical treatment of data

To identify the source(s) of possible between-years differences in the climatic and hydrological regimes, the relevant data were subjected to Principal Components Analysis (PCA). The analysis reduces the number of variables in the data set by extracting combinations (principal components or axes in n-dimensional space) of those variables that account for the highest proportions of variation (Poole, 1974). The analysis was run using the PCA routine of PRIMER® (Plymouth Routines In Multivariate Ecological Research; Clarke & Gorley, 2001) version 5.0 software.

The strength of associations between the biotic variables (seasonal log abundance and biomass anomalies) and those related to the lagoon's climate and hydrology was explored by calculating partial correlation coefficients between the biological and abiotic variables. To allow for the fact that responses of biological communities to changes in their environment involve time lags of various duration (Rudnick & Oviatt, 1986; Millet & Cecchi, 1992; Goedkoop & Johnson, 1996; Ólafsson & Elmgren, 1997; T. Radziejewska, unpubl. data), abiotic data averaged over periods of time representing <1, 1, 3 and 6 month lags were entered into calculations of partial correlations, except for the near-bottom water conductance values which pertained to time lag <1 month only. Statistics were performed with Statistica® version 5 software.

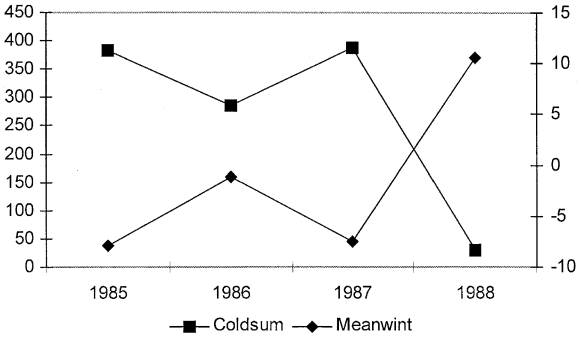

Figure 2. Cold sums (Coldsum; °C; left Y axis) and mean December–March temperatures (Meanwint; °C; right Y axis) of winters 1985–1988 in the Szczecin Lagoon area.

Results

Abiotic data

As shown by Fig. 2, the winters preceding each yearly sampling series differed widely in their harshness: while the winter 1984–1985 was severe and the subsequent winter (1985–1986) could be classified as normal, the winter 1986–1987 was severe again and was followed by an exceptionally mild winter 1987–1988.

The atmospheric circulation types prevailing in each month over 1984–1988 (Fig. 3a) varied from year to year, the most consistent pattern being shown mainly during autumn months (September–November), with air masses advecting predominantly from western and north-western sectors. The dominant atmospheric circulation types differed between the winters of the period of study: while the winter 1984–1985 showed prevalence of eastern circulations, circulation in the winter 1998–1986 was mainly from western sectors, advection from eastern sectors dominated in the winter 1986–1987, and both western and

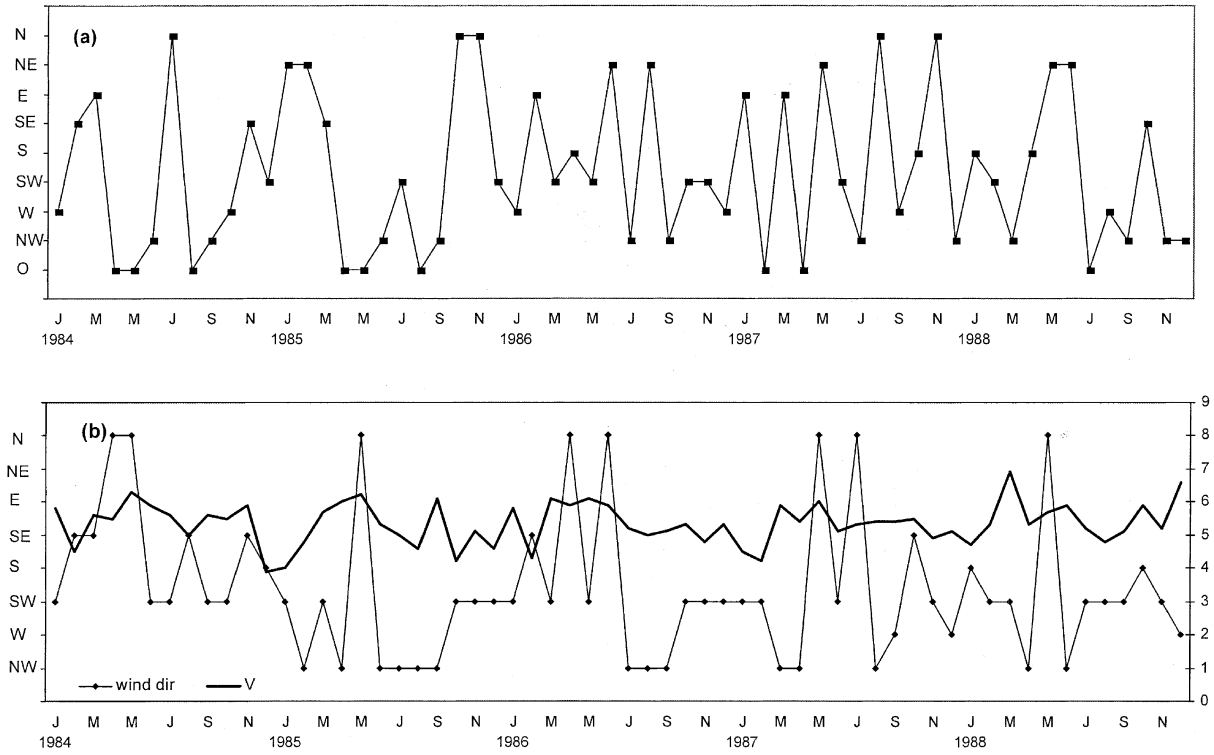

Figure 3. Variability of atmospheric circulation directions (a) over Poland as well as wind directions (wind dir; left Y axis) and speed (V; m s^{-1}; right Y axis) (b) in the Szczecin Lagoon area in 1984–1988.

southern sectors supplied the air masses advecting in the winter 1987–1988.

The prevailing winds (Fig. 3b) showed a more consistent pattern in that stronger winds tended to occur in the first half of each year, coincident with the prevalence of northerlies as well as south- and north-westerlies.

The mean monthly water levels and flow rates (Fig. 4) tended to show opposite patterns of change: low flows were usually accompanied by elevated water levels and *vice versa*. Except for 1985 with wide flow rate differences throughout the year, mean monthly flow rates tended to be high in the spring months, the flow slacking down considerably in summer and autumn.

Despite wide spatial variations, the mean near-bottom water conductance (Fig. 5) followed a consistent annual pattern, the conductances being markedly higher in the second half of the year, evidencing intensitifed intrusions of the Baltic Sea water into the lagoon. In 1986 and 1987, the mean conductances tended to be lower than those in 1985 and 1988.

The first three PCA axes (PC1, PC2 and PC3, respectively) together explained 75.2% of the variation in the climatic and hydrological data (Table 2). The winter severity-related variables (cold sum and mean winter temperature) weighted most heavily on PC1, while lagoon hydrography-related variables (flow rate and conductivity) were most important in separating the data along PC2. PC3 received the heaviest weighting from circulation type. However, as circulation type is known to affect the severity of winter (Girjatowicz, 2001), our PC1 and PC3 may be interrelated, for which reason a two-dimensional PCA plot (Fig. 6) is used to visualise the resultant principal component ordination. The ordination shows the year 1988 (mild winter) to be conspicuously isolated from the remaining data along PC1; the separation of data along PC2 reflects more the seasonal component of variation in the data set with seasonally varying flow rate and conductance (see above) than the interannual one.

Biotic variables

The lagoon's meiobenthos is invariably dominated by nematodes and ostracods (Radziejewska & Drzycimski, 1988, 1990). The variability in meiobenthic abundances had a strong spatial dependence, the

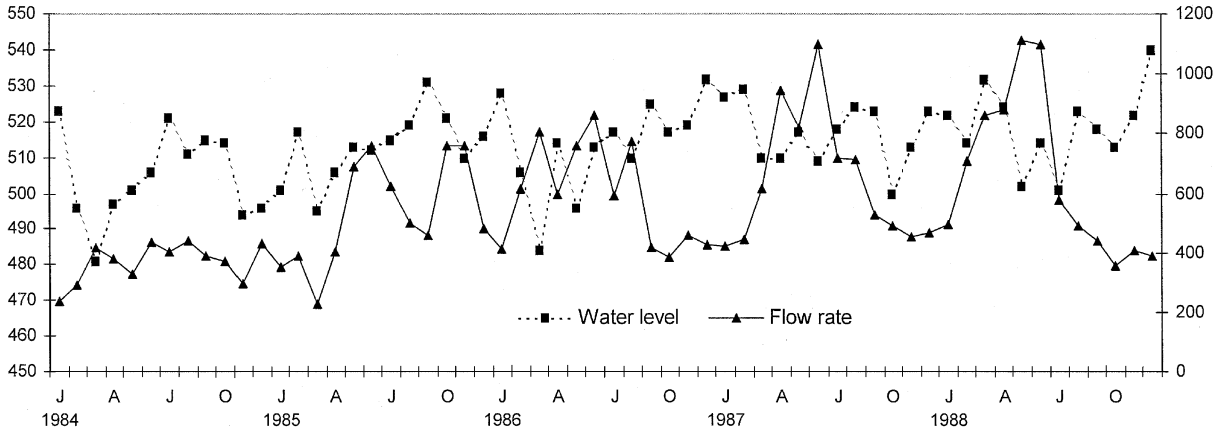

Figure 4. Monthly means of flow rates ($m^3\ s^{-1}$; left Y axis) and water level (cm; right Y axis) of the river Odra in Szczecin over 1984–1988.

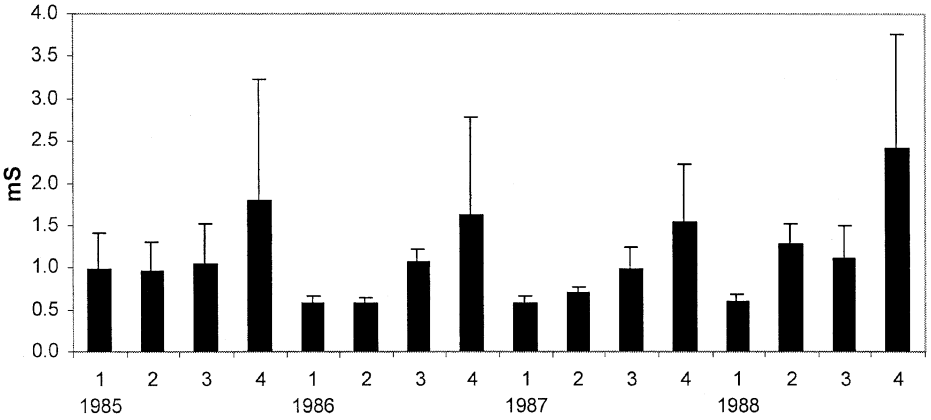

Figure 5. Mean basin-wide near-bottom water conductances in the Szczecin Lagoon sampling events in seasons (1–4) of 1985–1988.

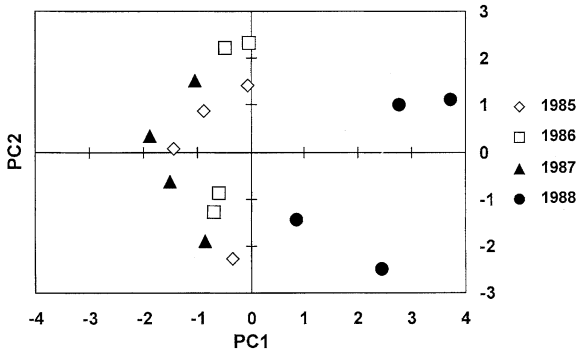

Figure 6. Two-dimensional PCA plot of climatic and hydrological variables.

highest abundances being found in the outer reaches of the lagoon (stations C and B in Fig. 1) and the lowest in the inner part (Radziejewska & Drzycimski, 1988). The signal from the interannual dimension was also pronounced. This is summarised by a plot of changes in the seasonal mean log abundance anomalies (Fig. 7a). These anomalies were positive in almost all seasons of 1985 and 1988 and negative in almost all seasons of 1986 and 1987. Meiobenthic seasonal log abundance anomalies did not correlate significantly, at any time lag, with any of the abiotic variables (Table 3).

The lagoon's macrobenthos is typical of predominantly fresh, eutrophic water bodies and is dominated by oligochaetes and chironomid larvae (Masłowski, 1992). Although the spatial component in the macrobenthic variability was pronounced, the abundances tending to increase towards inner parts of the estuary, the interannual part of the temporal component was very substantial (Masłowski, 1992). This is illustrated by Fig. 7b,c: the seasonal mean log anomalies of abundance and biomass were – similarly to those of the meiobenthos – positive in almost all seasons of 1985 and 1988 and negative (extremely so in 1987)

Table 2. Summary of Principal Components Analysis (PCA) results.

	PC1	PC2	PC3
% variation explained	29.5	27.8	17.9
Most important variables and their eigenvectors (in parentheses)	Cold sum (−0.553) + mean winter temperature (0.543)	Flow rate (0.500) + conductance (−0.477)	Circulation type (−0.515)

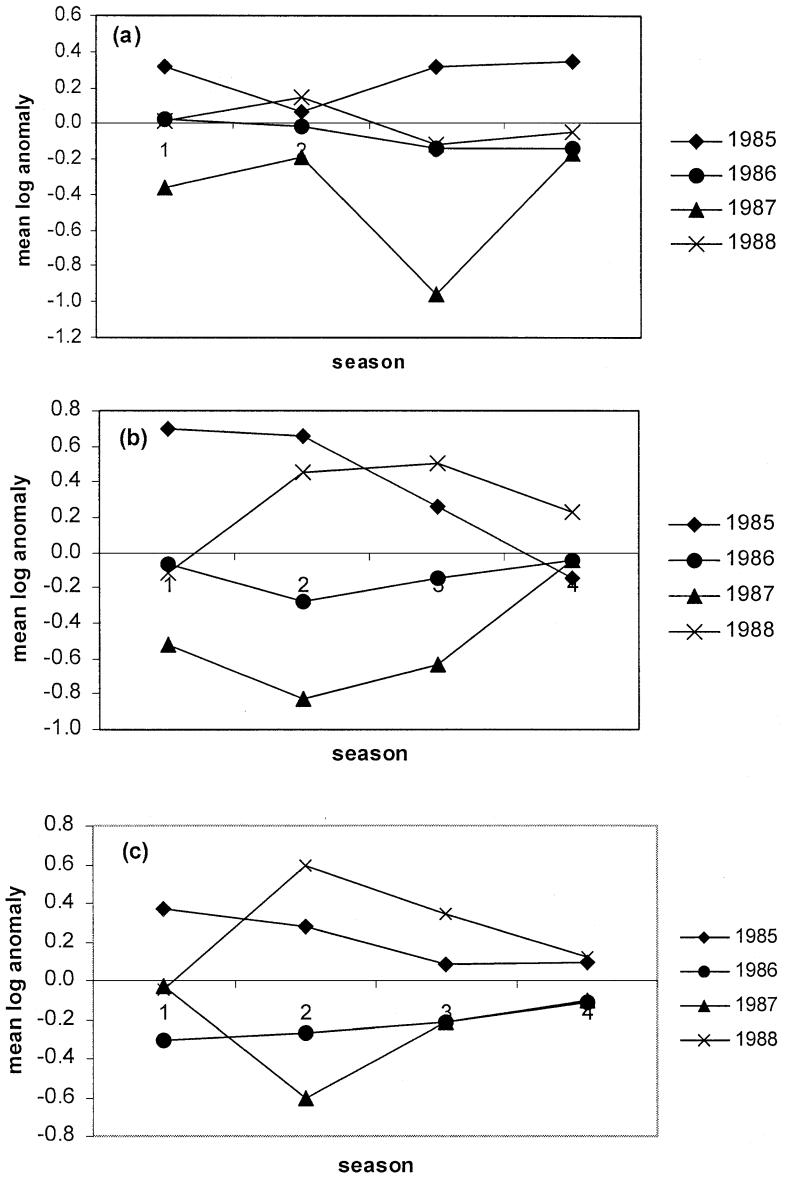

Figure 7. Seasonal mean log anomalies of meiobenthic abundance (a), macrobenthic abundance (b), and macrobenthic biomass (c). Error bars omitted for clarity.

Table 3. Summary of partial correlation coefficients between seasonal mean log benthic abundance (meio- and macrobenthos) and biomass (macrobenthos) anomalies vs. climatic and hydrological variables in the Szczecin Lagoon over 1985–1988.

Variable	Seasonal mean anomaly of											
	Meiobenthos abundance				Macrobenthos abundance				Macrobenthos biomass			
	Time lag (months)				Time lag (months)				time lag (months)			
	<1	1	3	6	<1	1	3	6	<1	1	3	6
Mean winter temperature	ns	ns	ns	ns	ns	ns	−0.76*	−0.65*	ns	−0.69*	−0.92***	−0.68*
Cold sum	ns	ns	ns	ns	ns	ns	−0.78*	−0.68*	ns	−0.71*	−0.92***	−0.71*
Circulation	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	0.85**	ns
Wind direction	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Wind speed	ns	ns	ns	ns	−0.75*	ns	ns	ns	ns	ns	ns	ns
Near-bottom water conductance	ns	–	–	–	ns	–	–	–	ns	–	–	–
Flow rate	ns	ns	ns	ns	ns	−0.70*	−0.76*	ns	ns	ns	−0.69*	ns
Water level	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	−0.68*	ns

*** = significant at $p < 0.001$; ** = significant at $p < 0.01$; * = significant at $p < 0.05$; ns = not significant.

in the two intervening years. Macrobenthic seasonal log abundance anomalies were found to be significantly (negatively) correlated with wind speed over time lag <1 month, with flow rate over 1-month time lag, with winter severity and flow rate over 3-month time lag, and with winter severity over 6-month time lag (Table 3). The lagoon's log biomass anomalies turned out to be significantly (negatively) correlated with winter severity over all time lags and with flow rate and water level over 3-month time lag, a positive correlation with circulation type being found for 3-month time lag (Table 3).

Discussion

The first question addressed in this study was whether climatic and hydrological conditions, concurrent or time-lagged, were involved in controlling the variability of the Szczecin Lagoon's benthic communities, observed during the four years of study. The analyses indicated that such controls could be operative, although their effects were neither straightforward, not readily discernible.

The abiotic parameters and variables used in this study were selected due to their known importance in shaping the meteorological and hydrological regimes of the water body investigated. As shown by the results of PCA and partial correlations (Tables 2, 3), the most important variables included the severity of winter as well as flow rate and the near-bottom water conductance. Moreover, it is worth noticing that the lagoon's meio- and macrobenthos responded differently to the same set of variables.

The degree of winter severity along the southern Baltic coast depends on atmospheric circulation (Girjatowicz, 2001), itself a process related to the NAO, the ultimate source of weather variability in winter over vast areas of Eurasia (Gerten and Adrian, 2001), including the southern Baltic coast (Marsz, 1999). As a rule, prevalence of eastern circulations in the winter months results in severe winters, while advection of air masses from western and southern sectors yields normal or mild winters (Girjatowicz, 2001). This general signal was clear in our data. The severity of winter was shown to be correlated with macrobenthic abundance and biomass anomalies (Table 3). Winter severity affects water temperature, the presence and persistence of ice cover (Girjatowicz, 2001), and the timing and intensity of the spring phytoplankton bloom (Townsend & Cammen, 1988; Fennel, 1999). The latter could be of importance for benthic populations as a source of labile organic matter sedimenting to the bottom (Graf et al., 1984; Christensen & Kanneworff, 1986; Kanneworff & Christensen, 1986). The major bloom in the lagoon, however, occurs later in the year (summer), in the form of mass development of the cyanobacterium *Microcystis aeruginosa* (Mutko et al., 1994), the sedimenting biomass of which increases the organic matter pool in the sediment (T. Radziejewska, pers. obs.) and perhaps fuels the extant populations. Their size and structure, however, should depend on their survival rates from one year to the next. As the correlations were significant for the macrobenthic abundance and biomass anomalies (Table 3), it seems that the severity of winter affected the macrobenthic populations indirectly, perhaps by influencing their

potential for growth later during the year. Although no study on the macrobenthic winter survival in the lagoon has been conducted to date, effects of winter harshness on the benthos are known from other areas (e.g., Beukema, 1991; Beukema et al., 1996; Honkoop & Beukema, 1997; Honkoop et al., 1998; Kröncke et al., 1998).

Our unpublished data showed macrobenthic abundances to be significantly correlated with mean wind speeds for the month of sampling, i.e., assuming a time lag <1 month. That could mean that the macrobenthic communities might be susceptible to physical effects in the form of surficial sediment disturbance (horizontal transport and resuspension) generated by the wind-induced waves and water circulation (cf. Arfi et al., 1993). Winds and the flow rate are the major agents of the lagoon's hydrodynamics (Robakiewicz, 1993) and, due to the shallow depths, their effects extend across the water column to the bottom. The physical disturbance thus produced is known to affect mainly benthic epifaunal communities (Kube et al., 1996). Although the macrobenthos in the lagoon is predominantly infaunal, it is concentrated mainly in the uppermost 5 cm (Kopczyński, 1998), and is, hence, susceptible to disturbance affecting that layer (Arfi et al., 1993). Disturbance is probably most intense during the first part of each year (cf. Fig. 3b). It could be important in seasonal changes in macrobenthic population sizes, rather than in interannual variability: in 1985, when the mean wind speeds were high throughout the year (Fig. 3b), the macrobenthic communities were generally more abundant than in the two subsequent years.

Physical disturbance effects can also be invoked when trying to explain the significant associations between seasonal mean log anomalies in macrobenthic abundance and biomass and abiotic variables (Table 3), the anomalies being time-lagged over 1 and 3 months. In their study on Texas estuaries, Montagna & Kalke (1992) showed the macrobenthos to respond positively to the freshwater inflow, owing to the prevalence of freshwater species the recruitment of which was enhanced during increased flow. Although most macrobenthic species in the Szczecin Lagoon were of freshwater origin as well (Masłowski, 1992, 1993), the negative association could have been caused by the prevalence of physical disturbance effects associated with increased flow, the effects being not strong enough, however, to induce instantaneous change.

Meiobenthic log abundance seasonal anomalies produced no significant correlation with any of the abiotic variables tested (Table 3). However, our unpublished calculations showed meiobenthic population abundances to be significantly correlated with the near-bottom conductance (partial correlation coefficient of 0.42) prevalent at the time of sampling, thus reflecting a direct effect of salinity. This is an effect well-known from other estuaries throughout the world (e.g. Heip et al., 1995, and references therein), but its cause(s) still remain unclear. In contrast to Montagna & Kalke's (1992) study, however, the Szczecin Lagoon's meiobenthos was significantly positively correlated with the flow rate (partial correlation coefficient of 0.31; T. Radziejewska, unpubl. data), which – in light of the disturbance effects shown for the macrofauna – is difficult to explain. The flow-induced physical sediment disturbance should have acted primarily upon the meiobenthos, but perhaps the instantaneous disturbance effects were rapidly alleviated by enhanced colonisation of the disturbed patches, as the considerable part of the meiobenthos was accounted for by such quasi-hyperbenthic species as the ostracod *Cypria ophthalmica* (Radziejewska & Drzycimski, 1988; T. Radziejewska, pers. obs).

Overall, the results of this study provide additional evidence to differences between responses of meio- and macrobenthos to abiotic conditions (cf. Radziejewska & Masłowski, 1997). The responses of the first are rapid and direct (no time lag, correlations involving abundance), those of the latter being delayed and rather indirect (time lags, correlations with abundance and biomass anomalies).

The second question posed for this study, namely if a four-year-long period is sufficient for drawing conclusions on more or less remote (indirect) controls on interannual variability of benthic abundances and biomass can be answered conditionally only. Such period of observations allowed to glimpse on possible links and controls, but to substantiate any claims regarding hydrological and – particularly – climatic controls, longer time series of data are desirable. In addition, when exploring longer data series, certain confounding factors such as eutrophication- and pollution-related effects ought to be taken into account (cf. Beukema et al., 1996).

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MODELLING

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Effects of land-rise on the development of a coastal ecosystem of the Baltic Sea and its implications for the long-term fate of ^{14}C discharges

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Key words: carbon flow model, ecosystem dynamics, brackish water, safety assessments, ^{14}C

Abstract

Due to the long half-lives of many radionuclides, safety assessments of nuclear waste facilities often need to consider the potential fate of radionuclides discharged to the environment in the future. In this study we explored the environmental fate of a hypothetical ^{14}C release from a nuclear waste repository to a Baltic Sea bay in 2000 years. This was accomplished by connecting spatially linked biomasses and metabolic rates from a carbon flow model of the ecosystem at the site today to predicted changes in geomorphology and water exchange regimes. The employed extrapolation method was selected as shoreline displacement due to land-rise and sea level changes is the main process that affects the development of the coastal ecosystem around the repository in the coming 10 000 years. The modelling results indicate that the ecosystem will go through changes in several ecosystem properties in the coming 2000-year period, e.g. a decreased rate of primary production and changed feeding preferences of the fish community. Also, a decreased total biomass is expected and an ecosystem change altering the balance between producers and consumers towards a dominance of benthic plants. The changes of the ecosystem structure and carbon dynamics will also influence the potential fate of future discharges of ^{14}C. We estimated an up to 1000 times higher ^{14}C concentrations in biota compared to today. However, due to radioactive decay and reduced total biomass in the receiving ecosystem, the proportion of accumulated radionuclides is expected to decrease. Although the modelling approach used in this study is associated with several sources of uncertainty, it provides a way to both qualitatively and quantitatively assess likely effects of future discharges of contaminants.

Introduction

In a safety assessment for a nuclear waste repository (SFR) in Sweden, an analysis of the fate of hypothetical radionuclide releases in the environment was performed. The assessment required a detailed description of the local biotic environment and predictions of its development over thousands of years in the future. This is discussed in the present paper.

The SFR-repository is embedded in bedrock under the seabed in the bay of Öregrundsgrepen in the Baltic Sea (Fig. 1). It stores low and intermediate level radioactive operational waste (Carlsson, 1998). ^{14}C is one of the most important radionuclides in SFR as a potential dose contributor to the local environment (calculated in Lindgren et al., 2001). The ^{14}C isotope is a radionuclide of considerable interest due to the quantities often found in nuclear low and intermediate level waste as well as for its high environmental mobility, and long half-life (Liepins & Thomas, 1988). It is highly bioavailable and is easily introduced to the food web through primary producing organisms (Cook et al., 1998).

Assessment of radioactive contaminants usually requires that time-spans of thousands of years must be considered, which is far longer than in most other assessments. The long time-perspective is due to the long half-lives of many radionuclides, and as potential releases from repositories may occur in a distant future (van Dorp et al., 1999) they are also expected to occur at low levels over very long periods of time (Klos et al.,

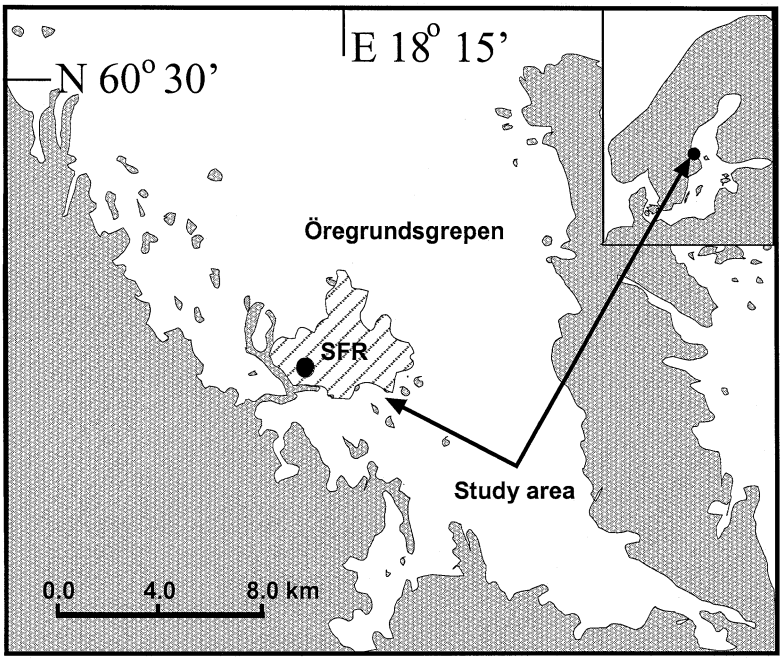

Figure 1. The study area and location of the final repository for radioactive operational waste (SFR) in a bay of Öregrundsgrepen, Baltic Sea (Sweden).

1999). From the SFR, a potential release of radionuclides can occur within the next 10 000 years (Lindgren et al., 2001).

The shoreline displacement is of main importance for the development of the coastal ecosystem including the area around SFR the coming 10 000 years, by transforming coastal ecosystems to freshwater and then to terrestrial environments (Brydsten, 1999a). The shoreline displacement is the combined effect of land-rise due to the release pressure after the past glaciations in Scandinavia, and due to the sea level rise caused by melting glaciers. The shoreline displacement can be estimated fairly accurately and predicted for the future (Påsse, 1997). Its implications on the underwater topography enable evaluations of the water exchange regimes, which will change with time (Engqvist & Andrejev, 2000). In modelling studies, the area around SFR was predicted to gradually transform from the open bay of today into a narrow archipelago in 2000 years, and then to lakes that will evolve to bogs and finally to meadows or forests (Brydsten, 1999a; Brunberg & Blomqvist, 2000; Jerling et al., 2000). This paper discusses the problem to scale and make a prediction of the ecosystem function in the bay in the coming 2000 years.

In the assessment of any contaminant, including radionuclides, distribution and transport models can be an essential component. They predict transfer pathways of the contaminant in the ecosystem and can identify organisms that may become exposed. By using flow models evaluating the carbon dynamics of the receiving ecosystem, the transport and fate of released ^{14}C may be predicted. In the safety assessment of the SFR, a carbon flow model was developed for the coastal ecosystem of today (Kumblad et al., 2003). In this paper that ecosystem model was used as the platform for the predictions of the expected carbon dynamics in the ecosystem in 2000 years.

The development of carbon flow models requires both mechanistic understanding of ecosystem properties, and site-specific data. A mechanistic description of ecosystems allows the identification of the main features and constraints of the ecological variables in the functioning ecosystem. With the help of the model, e.g. the possibilities for the ecosystem to maintain a mass balance or sustain the functional groups present can be assessed. However, there is a problem to obtain site-specific data for predictive modelling since ecosystems change considerably during the time span of thousands of years. Nevertheless, a scaling can be possible by associating site-specific ecological vari-

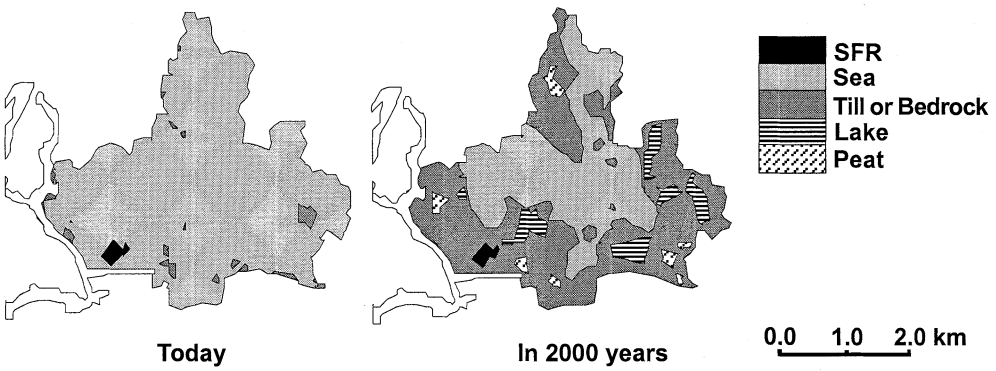

Figure 2. Map of the study area above the final repository of radioactive operational waste (SFR) in Öregrundsgrepen (Baltic Sea) today and in 2000 years including the type of land cover (modified after Brydsten, 1999a).

ables of existing ecosystems to changes in important abiotic parameters, e.g. temperature, salinity, nutrient concentrations, water flows or bathymetry. This is especially the case if the changes of the abiotic factors are better known and easier to predict than changes in the ecosystem itself. Sensitivity analysis of the mass balance and important variables and components of the model help to verify if the scaled model describes a realistic ecosystem including all essential components and processes.

In this study the major parameters that influence the change of the coastal ecosystem at the SFR were assumed to be the areal distribution of substrates at different depth intervals, the water volume, the water-turnover and the ecosystem structure and function found today at the site. The parameters were correlated to the land-rise process and to its implications on the water movement. In this paper the changing bathymetry and its effects on the water turnover is reflected in ecosystem properties and how the changes might determine the transport and fate of hypothetically released ^{14}C to the bay in 2000 years. The climate, nutrient load and composition of functional groups (but not the total biomass) were assumed to remain similar to those of today.

The objective of this study was (1) to explore the possibility of realistically scale the structure of future ecosystems with a documentation of the methodology, (2) to see if land-rise and changed water exchange regimes have an effect on the carbon dynamics, and (3) to estimate the ^{14}C concentration in biota after a hypothetical, future discharge from an underground, nuclear waste repository.

Description of the study area

Land rise

For the modelling exercise an area was chosen above the SFR in the Öregrundsgrepen of almost 12 km^2. It has a maximum and mean depth of 18 and 10 m respectively, experiences a rapid water exchange, and is for Baltic conditions fairly productive and species diverse. The Öregrundsgrepen area is subject to a substantial land-rise with a shoreline displacement of approximately 60 cm per 100 years (derived from Påsse, 1997). The land-rise will remain significant for the coming 10 000 years, altering the topography and proportion between land and water considerably. This process was modelled by Brydsten (1999a). In three thousand years from now the whole study area has risen above the sea level of the Baltic Sea and about 20 lakes will remain within the area. In five thousand years from now, the whole Öregrundsgrepen area will be land. The time-period around 2000 years from now was identified as a critical time for the study of radionuclide distribution within the coastal ecosystem. At that time the study area will still have contact with the open Baltic Sea but has transformed into an enclosed bay with a reduced and estuarine type water circulation (Engqvist & Andrejev, 2000) (Fig. 2). The sedimentation will be controlled by the water circulation (Brydsten, 1999a). Within the next 2000 years, the shoreline will be displaced 11 metres below its present position of today. The land to water proportion will increase from 2.5% to almost 55%, the water volume will decrease by two thirds, and the mean water depth will change from 10 m to 3 m (Brydsten, 1999a; Table 1).

Table 1. Depth distribution (km^2 per depth interval in meters) and water volume (km^3) of the study area today (2000 AD) and in 2000 years (4000 AD) (Brydsten, 1999, pers. commun.).

Depth interval (m)	2000 AD (km^2)	4000 AD (km^2)
Land	0.3	6.2
0–1	0.3	0.9
1–2	0.4	1.2
2–4	0.9	1.9
4–6	1.4	0.9
6–10	2.8	0.4
10–15	4.4	0
15–20	0.9	0
Seabed total	11.2	5.3
Surface total	11.5	11.5
Photic zone	5.9	5.3
Aphotic zone	5.3	0
Volume	0.1	0.02

Water exchange

Today, the model area is characterised by a rapid water exchange and therefore the seabed is dominated by erosion and transport bottoms with heterogeneous and mobile sediments (Mo & Smith, 1988). Small areas of rocky substrate occur close to the mainland and the islands (Sigurdsson, 1987). The water retention time of the larger bay Öregrundsgrepen is 12 to 26 days (surface to bottom waters) as an annual average, but is considerably quicker in the study area, 0.5 to 1.2 days (Engqvist & Andrejev, 1999). By using sensitivity analysis, the future water retention time in the area was estimated to approximately eight days as an annual average in 2000 years (Engqvist & Andrejev, 2000).

Biology

The seabed in the photic zone (above 10 m) is covered with microalgae and has comparably high species diversity and large amounts of macrophytes (Snoeijs, 1985, 1986, Kautsky et al., 1999). The grazing gastropod *Theodoxus fluviatilis*, the omnivorous crustacean *Gammarus* spp., and the filter feeding bivalves *Cardium* spp. and *Macoma balthica* dominate the phytobenthic macrofauna in the area (Kautsky et al., 1999). Seabeds below the photic zone have lower species diversity than the phytobenthic community, and are dominated by the detritus- and filter-feeding bivalve *Macoma balthica* (Kautsky et al., 1999). Major meiofauna taxa are nematoda, halacaridae, cladocera, copepoda and ostracoda (Snoeijs & Mo, 1987). Diatoms and dinoflagellates are the dominant phytoplankton during spring bloom, while cyanobacteria and small flagellates are frequent during the summer and autumn (Lindahl & Wallström, 1980). Two copepod species (*Acartina bifilosa* and *Eurytemora affinis*) constitute about 80% of the zooplankton biovolume and remaining 20% are cladocera, rotatoria, ciliata and larvae stages of benthic animals (Eriksson et al., 1977, Persson et al., 1993). Herring (*Clupea harengus*), roach (*Rutilus rutilus*) and perch (*Perca fluviatilis*) are the dominating fish species in the area (Neuman, 1982).

Ecosystem structure

The area is shallow and about half of the seabed is located in the photic zone. Consequently, more than half of the total biomass (53%) consists of organisms in the phytobenthic community, 39% are soft bottom organisms and 8% are pelagic (Kumblad et al., 2003).

Methods

The carbon flow model

The carbon flow model of the ecosystem in the area today, which is used as platform for this study, is described in detail in Kumblad et al. (2003). Components of the ecosystem in the study area were identified and their biomasses and annual rates of primary production, respiration, consumption and egestion (unassimilated food) were determined from *in situ* measurements and/or calculations from biomass data with the aid of conversion factors. Ambient annual temperature and light intensity was compensated for in calculations of the metabolic variables. Biomasses and metabolic rates, which were assembled at species level, were then merged to functional groups (Table 2). Instead of including several fish compartments into the model, an average biomass and consumption rate was based on the distribution of the fish species in the area (Neuman, 1982) and their main food sources (Curry-Lindahl, 1985), as fish have different feeding habits. The compartments were combined into a carbon flow model for the bay including the main components of the food web. White-tailed eagle, grey-seal, eider duck

Table 2. Description of the ecosystem components included in the carbon flow model and their assumed occurrence in the modelled system.

Compartment	Description
Phytoplankton	Phytoplankton (pelagic microalgae and photosynthesising bacteria; in the water column in the photic zone)
	Heterotrophic bacteria (pelagic bacteria; in the whole water column)
Zooplankton	Zooplankton (planktonic animals; in the whole water column)
Benthic plants	Microphytes (benthic microalgae; on the seabed in the photic zone)
	Macrophytes (benthic macroalgae, phanerogams, bryophytes; on the seabed in the photic zone)
Macrograzers	Macrograzers (grazing macrofauna >500μm; on the seabed in the photic zone)
Fish	Fish (demersal and pelagic; in the whole area)
Benthos	Filter feeders (filter feeding macrofauna > 500 μm; in/on the seabed at all depths)
	Benthic macrofauna (soft bottom dwelling macrofauna > 500 μm; in/on the seabed below the photic zone)
	Benthic meiofauna (meiofauna in/on the seabed 3–500 μm; in/on the seabed at all depths)
	Benthic microfauna (benthic bacteria <3 μm; in/on the seabed at all depths)
POC	Nonliving particulate organic carbon (in the whole water column)
DIC	Nonliving dissolved inorganic carbon (in the whole water column)

and humans were also included in the model since they were identified as interesting from a dose assessment perspective. The compartments depending on the water movement, i.e. phytoplankton (including heterotrophic bacteria), zooplankton, dissolved inorganic carbon (DIC) and particulate organic carbon (POC), were associated with the water exchange to enable estimation of import and export of carbon to and from the ecosystem.

In the model, carbon was considered to enter the food web through carbon fixation (from the DIC compartment) by primary producers and then transfer to higher trophic levels over grazers and predators. The respiration of carbon was associated with the DIC compartment providing a way for re-circulation of carbon within the ecosystem and egestion was linked to the POC compartment providing detritus feeding, benthic fauna with organic carbon.

Verification of the carbon flow model

The carbon flow model established for the present ecosystem (Kumblad et al., 2003) was compared with carbon budgets for adjacent areas in the Baltic Proper (Mc Kellar & Hobro, 1976; Jansson & Wulff, 1977; Ankar & Elmgren, 1978; Jansson et al., 1982; Larsson et al., 1986; Kautsky & Kautsky, 1995; Sandberg et al., 2000). The comparisons showed good agreement for both stocks and flows, although biomass deviations for benthic macrofauna (five times lover in this study) and for components of the pelagic system (lower in this study) were found. However, lower biomasses of these organisms groups have often been observed in the Bothnian Sea compared to the Baltic Proper (compilation in Kautsky & Kautsky, 1995).

Construction of the carbon flow model of the ecosystem in 2000 years

To assemble a separate carbon flow model for the ecosystem at the same location in 2000 years from now, biomass and metabolic rates for each functional group in the ecosystem at the site today were transformed to be applicable per square/cubic meter for different depth intervals (Table 1). The normalised data were then multiplied by estimated hypsographic data for the area in 2000 years (Fig. 3; Brydsten, 1999a) and the predicted water exchange rate for this period was also included (Engqvist & Andrejev, 2000).

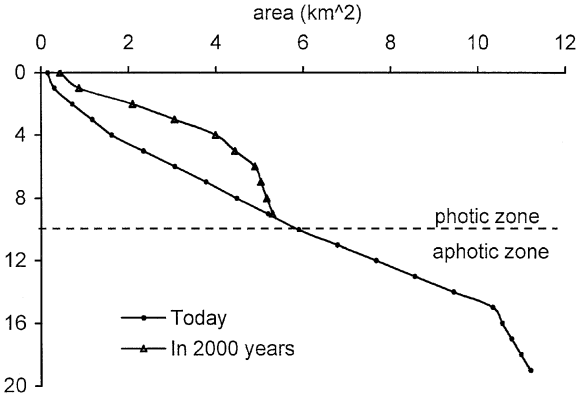

Figure 3. Hypsographic diagram of the study area above the final repository of radioactive operational waste (SFR) in Öregrundsgrepen (Baltic Sea) today and in 2000 years. Area (km^2) above depths (m) for the study area (modified after Brydsten, 1999a).

Assumptions of environmental properties in the area in 2000 years compared to today included that the ecosystem will contain the same functional organism groups in the environment (Table 2), and that these functional groups will remain in the same depth intervals and have the same abundance (biomass m^{-2}) within the depth intervals. It was also assumed that the light intensity and temperature will remain constant on an annual basis and that the water transparency will not decrease more than 50%. The concentrations of available DIC and nutrients in Öregrundsgrepen were also kept constant in the model.

Modelling of ^{14}C

In the model, the simulated ^{14}C discharge was introduced to the DIC compartment at a constant rate of 51.3 MBq per year. This corresponds to an estimate of the maximum annual discharge from the SFR-repository (Lindgren et al., 2001). ^{14}C released to the DIC compartment was introduced to the food web via accumulation by plants in proportion to the ^{14}C concentration in the DIC compartment and the rate of primary production. The subsequent transfer of ^{14}C in the food web was then modelled proportional to the flow of carbon. Thus, the ^{14}C concentration is dependent on the metabolic rates of the compartments (i.e. production, consumption and respiration), the ^{14}C concentration in the food sources or DIC (for plants), and the water exchange rate.

Results

Model predictions of ecosystem structure in the area in 2000 years

In 2000 years, the whole seabed is expected to be located within the photic zone if the water turbidity remains the same. As a consequence, 90% of the biomass in the area will be associated to the phytobenthic community, 7% to the soft bottom community and 3% to the pelagic (Fig. 4). The benthic plants (micro- and macrophytes) will dominate on biomass basis (85%) (Fig. 5). Of the remaining 15% of the total biomass, benthos constitutes 10%, macrograzers 3%, fish 2% and phytoplankton and zooplankton about 1%.

Analysis of the model mass-balance

In analysis of the mass-balance of the model, it was evident that primary production became limited by the DIC supply. To obtain a realistic estimation of the primary production, nutrient dynamics was included in the model. The nutrient uptake by primary producers was assumed to be proportional to the stoichiometric relationships between carbon and the nutrients (i.e. Redfield ratios) and the trophic transfer of the nutrients was assumed to be proportional to the mass flow of carbon. The nutrient mineralization by consumers was assumed to be proportional to respiration and estimated excretion ratios (Kautsky, 1995a). Initial nutrient concentrations available for primary production were assumed to be the same as average nutrient concentrations in the area today (Nitchals, 1985; Lindblad, 1994). The combined carbon and nutrient modelling lead to a calibration of the model in terms of a reduced primary production. The total primary production still exceeded the total consumption after this calibration. This indicates that the estimated plant biomass can be maintained with the reduced rate of primary production.

The mass-balance analyses also indicated that the zooplankton community would crash due to a too high predation pressure from fish. This may be a consequence of decreased phytoplankton primary production on which the zooplankton stock relies. Consequently, the model was calibrated by re-adjusting the food selection for fish to become proportional to the abundance of their preys. The mass balance was tested again and a viable zooplankton and fish population was possible to maintain by changing the feeding preferences for fish from 80% zooplankton, 10% benthic plants, 5% each of macrograzers and benthos to 20%

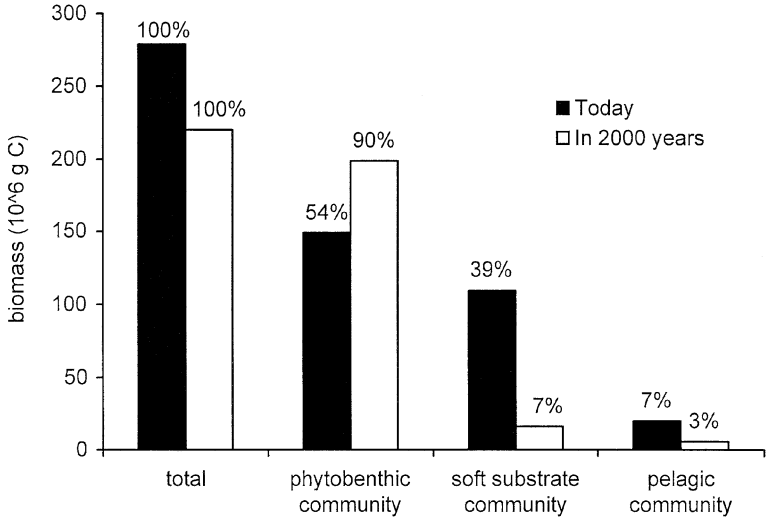

Figure 4. Amount (10^6 g C) and proportion within each period (%) of total biomass between communities in the study area above the final repository of radioactive operational waste (SFR) in Öregrundsgrepen (Baltic Sea) today and in 2000 years. The phytobenthic community comprises benthic plants, macrograzers, filter feeder, benthic micro- and meiofauna, the soft substrate community filter feeders, benthic macro-, micro- and meiofauna, and the pelagic community plankton and fish.

zooplankton, 10% benthic plants, 10% macrograzers and 50% benthos.

The results presented in this paper originate from the calibrated version of the model. Comparisons are made with the model of the present ecosystem (extracted from Kumblad et al., 2003).

Distribution of biomass: a comparison with the present ecosystem

Due to the shoreline displacement the whole area will become shallower and the total surface of the seabed is expected to decrease from 11.2 km^2 to 5.3 km^2 within the next 2000 years. This result in both a decrease of the total biomass of about 20%, and a larger dominance of biomass associated to the phytobenthic community (90%) compared to the area today (54%) (Fig. 4).

The biomass distribution between producers and consumers will change from equal occurrence on a biomass basis in the present ecosystem (Kumblad et al., 2003) to an environment totally dominated by plants (86%) in 2000 years (Fig. 5).

The distribution of biomass between the functional groups appears to be heterogenic in both models (Fig. 5). In the present ecosystem, the benthic plants and the benthic macrofauna dominate and make up more than 40% each of the total biomass. In 2000 years, the benthic plants alone will increase to 85% at the cost of soft bottom consumers (10%), especially *Macoma balthica*.

Carbon fluxes: a comparison with the present ecosystem

The studied area is self-sufficient with regard to organic carbon and it will still be in 2000 years. That is, the total biomass production is larger than the total consumption, which causes a net export of organic carbon from the area. Today, the annual export of POC from the area makes up about 10% of the total annual primary production. This export will decrease to 3% in 2000 years (Fig. 6). Benthic plants contribute to 73% of the total carbon fixation, which is expected to decease to 56% in the future (Fig. 6).

^{14}C concentrations in biota: a comparison between the fate of a hypothetic discharge today and in 2000 years

Modelling results indicate that many flows and storages of carbon will change considerably during the 2000-year period considered in this study owing to various environmental and biological factors. These changes will also influence the fate of ^{14}C if released to the ecosystem. The expected ^{14}C concentrations in biota, DIC and POC would increase up to almost a thousand-fold if there would be a discharge in 2000 years compared to today (Table 3). The ^{14}C concen-

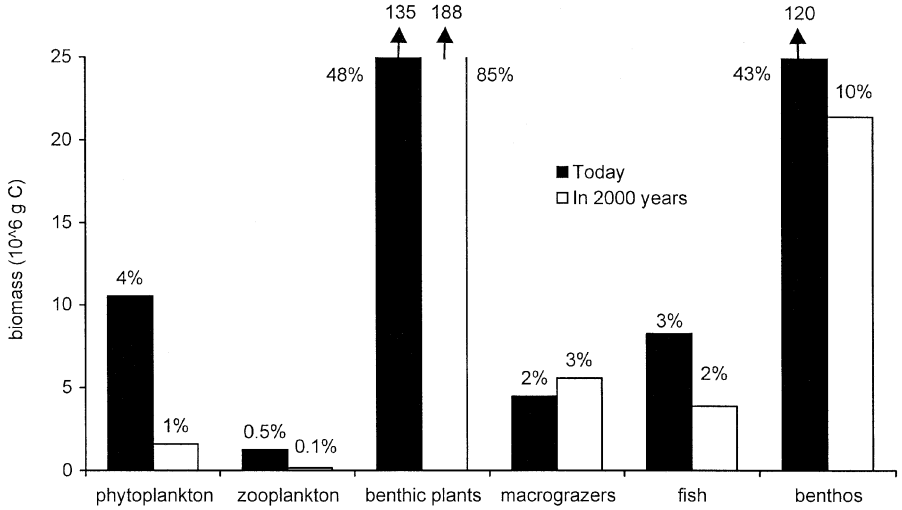

Figure 5. Amount (10^6 g C) and proportion within each period (%) of total biomass between compartments in the study area above the final repository of radioactive operational waste (SFR) in Öregrundsgrepen (Baltic Sea) today and in 2000 years.

Table 3. Steady-state concentrations of ^{14}C in biota, POC (μBq g C^{-1}) and DIC (μBq l^{-1}) from a constant annual discharge of 51.3 MBq $year^{-1}$ to the ecosystem above the SFR-repository today (2000 AD) and in 2000 years (4000 AD).

	2000 AD μBq g C^{-1} (l^{-1})	4000 AD μBq g C^{-1} (l^{-1})
DIC	1.3	70
POC	5.7	2400
Phytoplankton	5.7	860
Zooplankton	0.47	350
Benthic plants	67	2400
Macrograzers	62	2200
Fish	9.1	1400
Benthos	5.1	2200

trations in biota mirror their location in the food web and their metabolic rates.

The highest ^{14}C concentrations both today and in 2000 years were found in benthic plants (67 μBq g C^{-1} and 2400 μBq g C^{-1} respectively). This is a result of their fixation of carbon (and ^{14}C) directly from the DIC compartment, to which the ^{14}C is discharged. Phytoplankton also accumulates ^{14}C through this mechanism but get about ten to three times lower concentration than benthic plants in the present ecosystem and in 2000 years respectively (5.7 μBq g C^{-1} and 860 μBq g C^{-1}). This is the consequence of a dilution effect caused by the rapid water exchange that continuously replaces large fractions of the contaminated with uncontaminated phytoplankton. Zooplankton gets even lower ^{14}C concentrations than phytoplankton (0.47 μBq g C^{-1} and 350 μBq g C^{-1}). This is because they are both directly affected by the water exchange themselves as well as indirectly through their food source (phytoplankton). The ^{14}C concentrations in macrograzers (62 μBq g C^{-1} and 2200 μBq g C^{-1}) are almost as high as in benthic plants since they feed on benthic plants and are not affected be the water exchange. In the model of the present ecosystem, the fish to a large extent consume zooplankton. Consequently they obtain a low ^{14}C concentration (9.1 μBq g C^{-1}) compared to e.g. macrograzers and benthos. However, a ^{14}C discharge to the area in 2000 years would cause higher ^{14}C concentration in fish (1400 μBq g C^{-1}). This is a result of the changed food preferences for fish and the lower water exchange rate.

The ^{14}C concentrations in zooplankton, benthos and POC due to a ^{14}C discharge to the ecosystem in 2000 years compared to a discharge to the present ecosystem increases markedly more than the other compartments. The increase in zooplankton and POC is probably due to the lowered water exchange rate. The reason for the increase in benthos is probably a combined effect of the water exchange (since they consume POC) and the strongly reduced biomass for benthic organisms.

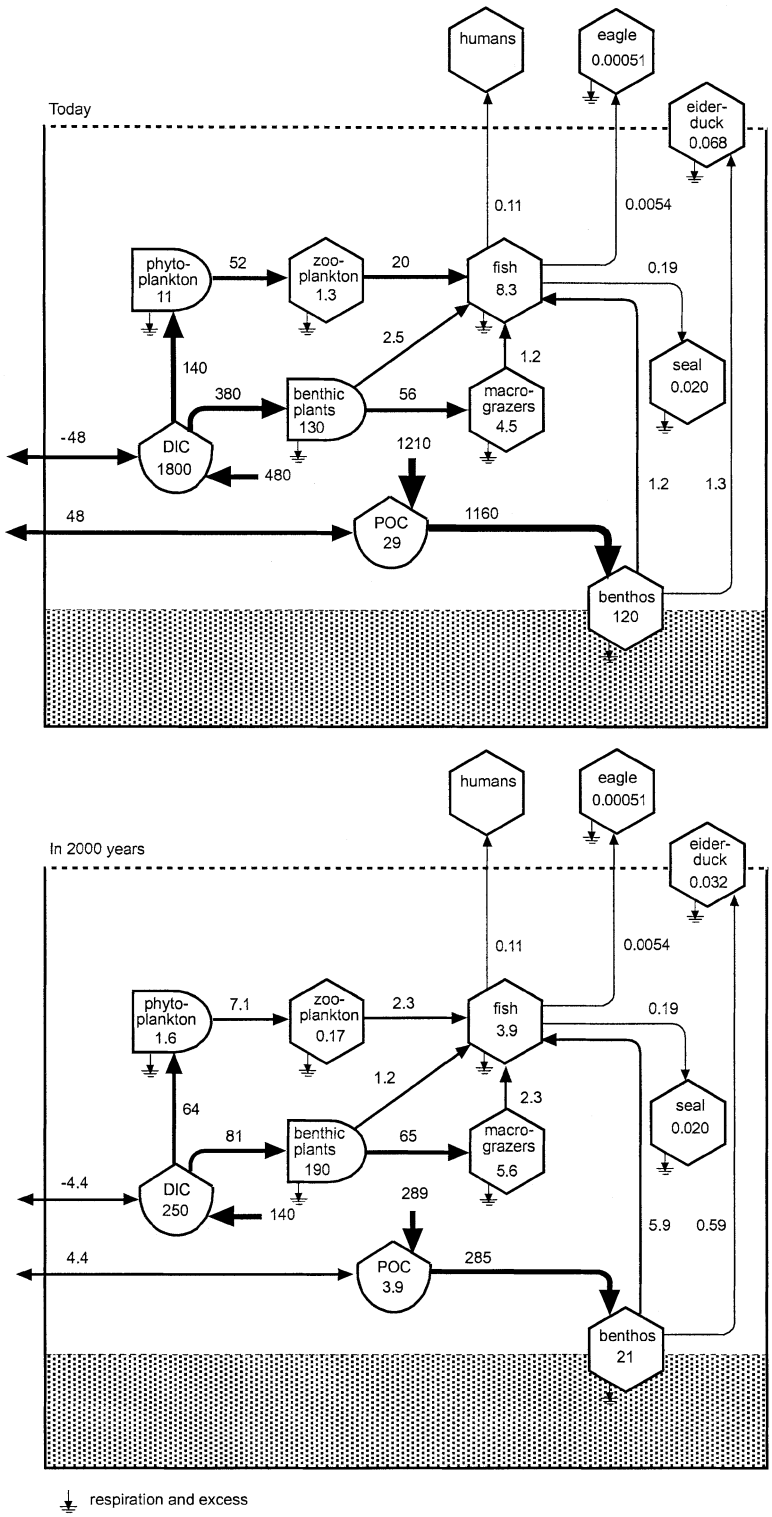

Figure 6. Distribution (10^6 g C) and annual flux (10^6 g C year^{-1}) of carbon in the study area above the final repository of radioactive operational waste (SFR) in Öregrundsgrepen (Baltic Sea) today and in 2000 years.

Analysis of expected changes of ecosystem structure and functioning and its implications on a hypothetical ^{14}C discharge

If the major structuring factors during the following 2000-year period are shoreline displacement and lowered water exchange rates, the ecosystem will probably go through moderate changes in ecosystem functioning.

When the availability of DIC and nutrients were kept at the same levels as prevailing in the present ecosystem, a limitation of primary production was forecasted in mass-balance analysis of the model. This indicates that the biomass of primary producers was overestimated and/or that a shift to a species composition with lesser carbon and nutrient demands and lower primary production rate can be expected, e.g. a species shift to more vascular plants (Kautsky, 1995c).

We expect increasing sedimentation as a consequence of decreased water turnover in the model area in 2000 years. This would increase the proportion of soft substrates in the area that probably would favour vascular plants. A resuspension of particles will also reduce the transparency of the water column. However, even at a substantial decline of the compensation depth, from e.g. 10 to 4 m, more than 75% of the seabed would remain in the photic zone since the future area is shallow (Fig. 3). Thus, the estimated biomass distribution of functional groups will not be influenced by a possible, realistic decrease in the water transparency. An increased sediment load in the deeper parts of the ecosystem could either stimulate soft bottom fauna due to more organic carbon for consumption or cause anoxic conditions due to increased mineralization of deposited material.

The zooplankton crash predicted by the model may be interpreted either as an effect of underestimating of the zooplankton production, overestimating the fish stock or the fish consumption rate, or as an altered diet of the fish community. The zooplankton production relies on the phytoplankton primary production. As the primary production rate is predicted to decrease as a consequence of nutrient limitation, there is no carbon available to increase zooplankton productivity.

To maintain the fish stock in 2000 years according to mass-balance analysis, the fish community must change food preferences to a higher dependence on benthic macrofauna instead of on zooplankton. In the present ecosystem herring is dominating the fish community. Herring is mainly zooplanktivorous and may consume a considerable proportion of the zooplankton production (Arrhenius & Hansson, 1993). Thus, a decreased supply of zooplankton can be interpreted as we can expect a decrease of the herring population and that fish species with more variable food preferences will become more abundant, e.g. pike and perch.

In the time frame of 2000 years, changes in other abiotic parameters such as temperature, salinity, nutrient load and introduction of alien species may also influence the development of the ecosystem. Irrespective of changes of the global average temperature, the modelled bay will probably experience a higher seasonal variation in temperatures due to lower water turnover and decreased water depth. This may enhance the metabolic turnover of carbon and nutrients in summer time and vice versa in winter. However, as observed from the mass balance analysis, large changes of e.g. total primary production and biomasses will probably not occur as the system is constrained by the external input of nutrients and dissolved inorganic carbon. The inter-annual temperature variation in the region will probably exceed potential changes of the long-term average temperature. This makes it reasonable to assume that the species composition in the bay will not be significantly affected by a small increase in annual average temperature.

The species distribution in the Baltic Sea reflects the north-south salinity gradient, with marine species gradually disappearing from the Swedish west coast northwards to the Bothnian Bay, and the opposite for the freshwater organisms (Segerstråle, 1957; Wallentinus, 1991; Kautsky, 1995b). Probably would changes in salinity shift the ecosystem structure to be similar to areas either located just north or south of the modelled bay. For instance, an increase in salinity would probably favour the occurrence of the filter-feeding blue mussel *Mytilus edulis* in the area, which is almost absent in the area today (Kautsky et al., 1999). This could significantly alter the function of the system by consumption of phytoplankton, which would compete with the pelagic grazing food chain. A slightly lower salinity will however not affect the modelled coastal ecosystem dramatically, because communities similar the present in the SFR-area are found far north in the Bothnian Sea where the salinity is lower (Kautsky, 1995b).

The effect of succession, eutrophication (Hansson & Rudestam, 1990), introduction of new species (Olenin & Leppäkoski, 1999) and human interactions on the environment are difficult to predict and was not possible to consider in this study.

The overall circulation and distribution of radionuclides in the environment is determined by the flow of energy in the receiving ecosystem (Whicker & Schultz, 1981). This is particularly applicable for C-14. The expected fate of hypothetically ^{14}C discharges into the ecosystem above the SFR repository would be different for a discharge today compared to in 2000 years. A future discharge would probably result in higher concentrations in the biota compared to today, because a lower water turnover and water volume causes a higher water concentration and a decreased export of radionuclides from the system. However, the total proportion of radionuclides accumulated in the biota could also be lower due to the reduced total biomass. Moreover, ^{14}C has decayed by about 35% during the 2000-year period. A large fraction of the erosion bottoms in the study area of today will shift into accumulation bottoms during this period. This may result in an accumulation of ^{14}C in the sediments (Brydsten, 1999b). The model simulations in this study indicate that the expected ^{14}C concentrations in biota due to discharges of 51.3 MBq per year are not likely to harm biota.

Conclusions

This study illustrates a possibility to retain realistic ecosystem structures and functions when re-scaling ecosystem models in proportion to changes of abiotic parameters to a future environment. It seems to be possible to re-scale a present-day ecosystem model into a new model that realistically describes the expected properties of a future ecosystem. The extrapolation might be accomplished by extrapolation of spatially linked biomass and metabolic rates to predicted geomorphologic data for the area in the future. The dynamic modelling performed in this study inspires to many new questions about the linkage of structure and function, both in future ecosystems as well as in existing ones. The implemented modelling approach is associated with many uncertainties but still provides a way to both qualitatively and quantitatively assess likely effects of discharges of contaminants in the future.

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INVASIVE SPECIES

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Distribution, population structure and salinity tolerance of the invasive amphipod *Gmelinoides fasciatus* (Stebbing) in the Neva Estuary (Gulf of Finland, Baltic Sea)

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Key words: Baikalian amphipod, Neva Estuary, seasonal dynamics, population structure, fecundity, salinity tolerance

Abstract

In the early 1970s, the Baikalian amphipod *Gmelinoides fasciatus* (Stebbing) was intentionally introduced into several lakes in the Gulf of Finland basin in order to enhance fish production. By 1996, *G. fasciatus* successfully colonized the littoral zone of Lake Ladoga and, via the Neva River, invaded the Neva Bay, the freshwater part of the Neva Estuary. In 1999, *G. fasciatus* was first registered in the inner Neva Estuary, the very first record of the Baikalian amphipod in brackish waters of the Baltic Sea. Distribution, abundance, reproduction and population structure of *G. fasciatus* in the Neva Estuary were studied during 1998–2000. In some locations of the Neva Estuary, maximum densities of *G. fasciatus* reached 3500 ind. m^{-2}. In general, density and biomass of *G. fasciatus* in the freshwater part of the Neva Estuary were higher (around 1.5 fold) than in the brackish-water part. Fecundity of this amphipod averaged 10–20 eggs per female, depending on body size of females and season. In order to assess the possibility of further spread of *G. fasciatus* in the Baltic Sea, the salinity tolerance of this species was determined in a series of laboratory experiments. Our results showed that the invasive amphipod *G. fasciatus* is potentially able to colonize shallow coastal habitats of, for example, the Gulf of Bothnia, Gulf of Riga and other parts of the Baltic Sea with water salinities ranging from 1 to 5 psu.

Introduction

Gmelinoides fasciatus (Stebbing) is a gammaridean amphipod of Baikalian origin, which before the late 1960s was restricted to freshwater ecosystems of East Siberia, including the Lake Baikal region (Lake Baikal and upper Angara River) and the Yenisei River basin (Bazikalova, 1945; Bekman, 1962). *G. fasciatus* was described first as *Gammarus zebra* for its distinct striped colour pattern (Dybowsky, 1874), and the 'zebra amphipod' can be considered an appropriate common name for this species.

In the 1970s, *G. fasciatus* was intentionally introduced into several lakes of the Karelian Isthmus (Baltic Sea basin) in order to enhance fish production (Nilova, 1976; Arkhiptseva et al., 1977). From these lakes, *G. fasciatus* spread into Lake Ladoga (Panov, 1996) and then, via the Neva River, invaded the Neva Estuary (Gulf of Finland). In the Neva Bay, the freshwater part of the estuary, the zebra amphipod was detected for the first time in 1996 (Alimov et al., 1998). In 1999, *G. fasciatus* was first registered in the brackish-water reaches of the estuary (Panov et al., 1999). Establishment of this invasive species in the Neva Estuary may result in significant alterations of littoral communities, as has occurred already in some lakes and reservoirs of Europe and Siberia (Panov & Berezina, 2002).

The aim of the present work was to study the distribution and population structure of *G. fasciatus* in the Neva Estuary, plus the salinity tolerance of the species in order to assess the present state of the population in the estuary and the possibility of further spread of this species in the Baltic Sea.

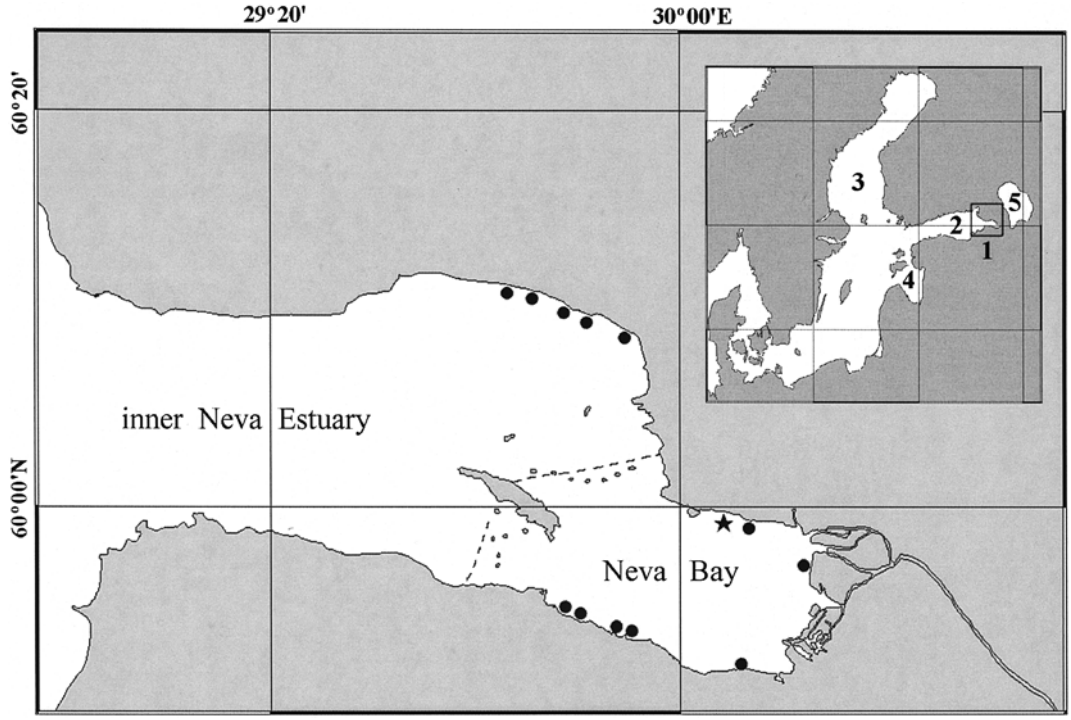

Figure 1. Distribution of the Baikalian amphipod *Gmelinoides fasciatus* in the Neva Estuary in 1998–2000. Asterisk shows the site of period of observations. The dashed line indicates the storm-surge barrier. Numbers in the inserted map of the Baltic Sea region: (1) Neva Estuary, (2) Gulf of Finland, (3) Gulf of Bothnia, (4) Gulf of Riga, (5) Lake Ladoga.

Materials and methods

Site descriptions

The Neva Estuary is the largest estuary in the Baltic Sea, the catchment area of the Neva River exceeding 280 000 km^2. The estuary consists of three main parts: the Neva Bay (400 km^2) and the inner and outer estuary (3200 km^2; Pitkänen, 1991). Our research was focused on the littoral zone (at depths from 0.2 to 1.5 m) of the Neva Bay and the northern part of the inner Neva Estuary (Fig. 1). The Neva Bay is a freshwater part of the estuary (salinity 0.06–0.30 psu). In the inner estuary, separated from the Neva Bay by a storm-surge barrier, the surface waters are brackish (0.6–2.0 psu). Description of the temperature and salinity regimes in the Neva Estuary is provided by Panov et al. (1999). In the littoral zone of the Neva Estuary, the coarse sand, gravel and stones are typical bottom substrates. Also, because of eutrophication, extensive macrophyte beds (mainly of reed *Phragmites australis* (Cav.) and bulrush *Scirpus lacustris* L.) develop during the summer in the littoral zone of the Neva Bay (Panov et al., 2002). In some locations, primary sand and gravel substrates are completely covered by macrophyte roots and debris, which provide suitable habitats for aquatic invertebrates. In the inner Neva Estuary, mats of the filamentous green alga *Cladophora glomerata* (L.), growing on hard substrates, represent the main habitat for invertebrates.

Fieldwork

Extensive biological summer surveys of the littoral communities of the Neva Estuary were conducted in 1998–2000. Also, samples of *G. fasciatus* were taken weekly from 25 May to 23 October 1998 in one location in the Neva Bay (Fig. 1). In the macrophyte beds, quantitative sampling was carried out with a cylindrical 0.125 m^2 corer (Panov & Pavlov, 1986) in two replicates. On hard substrates, quantitative samples were collected by SCUBA-divers, using 0.25 × 0.25 m metal frames in three replicates. The samples were preserved in 4% formaldehyde solution and transported to the laboratory in plastic bags. All collected amphipods were counted, measured and weighed (wet weight). Density, biomass, fecundity, size and sexual structure of the population of

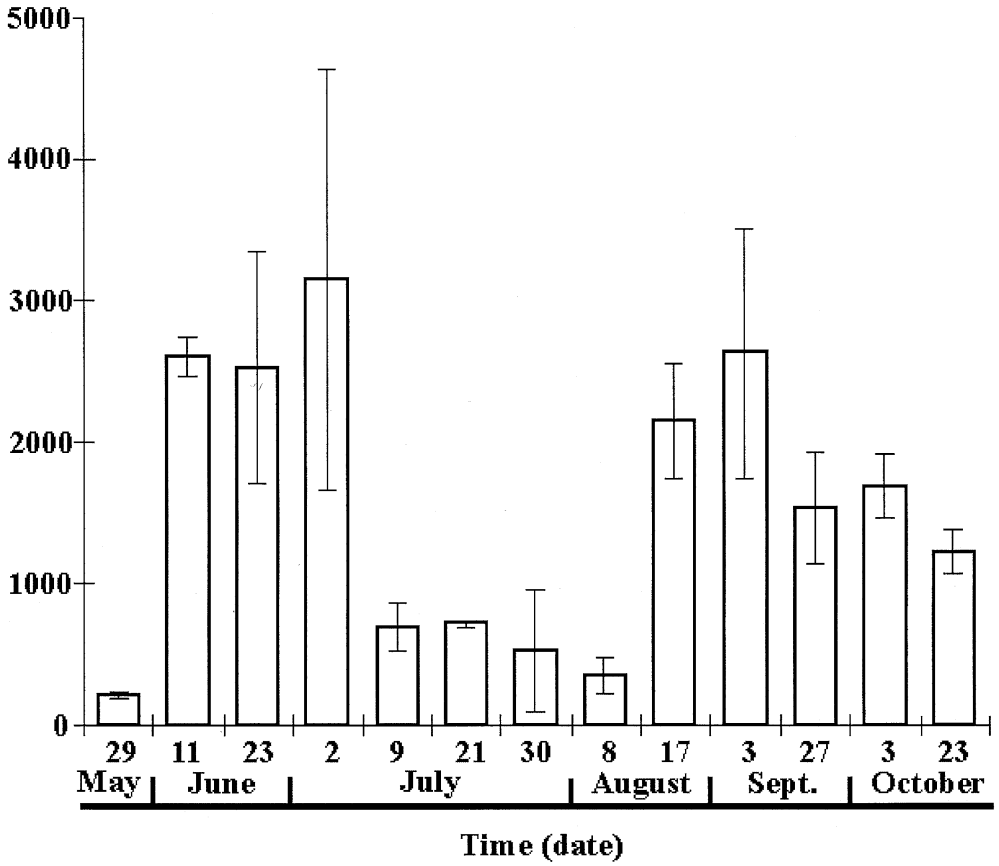

Figure 2. Seasonal dynamics of *Gmelinoides fasciatus* (mean density ± SE) in the littoral zone of the Neva Estuary in 1998.

G. fasciatus were evaluated. Density and biomass of amphipods were expressed as mean (SE: standard error).

Salinity tolerance of *G. fasciatus* at different water salinities and temperatures was determined in a series of laboratory experiments. Experiments were performed with sexually mature males with body length in the range 7.9–9.5 mm (mean 8.7 mm), sexually mature females (5.1–7.0 mm; mean 5.8 mm) and juveniles (2.6–3.3 mm; mean 2.9 mm), which were collected in the freshwater part of the Neva Estuary. The salinity tolerance of *G. fasciatus* was assessed as the survival rate of specimens transferred for 15 days from freshwater to water of different salinities (1–16 psu) and constant temperature (18 °C). The salinity was varied either abruptly (direct transfer) or in 1 psu increments at two-day intervals (stepwise adaptation; see Karpevich, 1968) or at ten-day intervals (stepwise acclimation; see Khlebovich & Kondratenkov, 1973). Also, we studied the development of eggs in females at different water salinities. Sexually mature females (body length 5.7–6.5 mm) and males of *G. fasciatus* were held together at 0.06 psu (control), and at 1, 2, 3, 5, 8, 10, 12 and 16 psu and water temperature of 18 °C. After fertilization, males were removed and fecund females were regularly examined. A separate series of experiments was conducted in order to evaluate the effects of temperature on salinity tolerance in *G. fasciatus*. The amphipods (only males) were held in experimental microcosms (volume 40 l) with various combinations of temperature and salinity (Table 3) for 30 days, before estimations of the coefficients of salinity tolerance. For estimations of these coefficients, the lethal salinity was evaluated after transfer of animals from microcosms to water with gradually increasing salinity (1 psu every 10 min). The value for the control variant (see Table 3) was taken for 1, and then the relative values of lethal salinities for other experimental variants were counted and considered as the coefficients of salinity tolerance for *G. fasciatus*.

The experimental water solutions were prepared by diluting artificial sea water (32 psu) with freshwater

Table 1. Average density, biomass and fecundity of *Gmelinoides fasciatus* in the littoral zone of the Neva Estuary.

Part of the estuary	Density, ind. m^{-2}	Biomass, g m^{-2}	Fecundity, eggs per female
Brackish water	1100 ± 700	2.95 ± 1.50	12.47 ± 2.12
Freshwater	1550 ± 1050	3.75 ± 3.00	12.67 ± 3.96

from the Neva River. The total mineral content of the Neva water was 0.05–0.06 psu. The salinity of experimental solutions was determined with an ATAGO S-10 refractometer to the nearest 0.05 psu. The water in the experimental vessels was changed every three days. Experiments were performed in triplicates. The oxygen concentrations in the water ranged from 7.2 to 8.6 mg l^{-1}. Amphipods were kept in 200-ml flat vessels and fed on a mixture of algae (mainly *Cladophora glomerata*), aquatic moss (*Drepanocladus* spp.) and dried birch leaves (*Betula* spp.).

Results

Distribution and population structure of G. fasciatus in the Neva Estuary

Results of the field surveys in 1998–2000 indicate that *G. fasciatus* established permanent populations in the littoral zone of both freshwater and brackish-water parts of the Neva Estuary (Fig. 1). During the reproductive period from May to October, most of the *G. fasciatus* population concentrates in the shallow near-shore habitats at depths less than 3 m. Maximum density of the zebra amphipod in the littoral zone of the inner Neva Estuary exceeded 1600 ind. m^{-2}. In some locations of the Neva Bay, maximum densities of *G. fasciatus* reached 3500 ind. m^{-2}. Average density and biomass of *G. fasciatus* in the Neva Bay were higher (around 1.5 fold) than in the brackish-water part of the estuary (Table 1).

Our results showed that *G. fasciatus* in the Neva Estuary possesses a one-year life cycle with 2 peaks in density during the season in 1998 (Fig. 2). Significant increase in density of the amphipod population, up to 3400–4800 ind. m^{-2} in late June and early July (first peak), was attributed to the appearance of large numbers of juveniles. The midsummer period was characterized by comparatively low population density (500–800 ind. m^{-2}), a decline in the number of overwintering adults and domination by immature females of the first generation (Fig. 3). Beginning in the first week of August, amphipods of the first summer generation became mature and started to reproduce, which resulted in the second peak of amphipod density up to 2800–3800 ind. m^{-2} in mid-August and September (Fig. 2). Fecund females were present in the *G. fasciatus* population until late September (Fig. 3). Thus, reproduction of the zebra amphipod in the shallow littoral zone of the Neva Bay started in 1998 in early spring at temperatures of 4–5 °C and terminated in October at water temperatures below 10 °C.

The fecundity of *G. fasciatus* averaged 8–36 eggs per female, depending on female body size (Fig. 4). During the reproductive period, from May to September, the mean size of fecund females and their fecundity decreased. In early July, their mean fecundity averaged 20 eggs per female, in early September it decreased to 10–11 eggs per female, and during this period mean sizes of females decreased from 7.3 ± 1.5 to 5.9 ± 1.1 mm.

Experimental study of salinity tolerance in G. fasciatus

After direct transfer from freshwater, during 15 days all males of *G. fasciatus* survived at salinities from 1 to 4 psu, while females were more resistant, tolerating salinities up to 6 psu. The salinity for 100% mortality was 13–16 psu for males and 15–18 psu for females. The salinity tolerance in juveniles was significantly lower, 50% of them dying within one day at 4–5 psu. Their mortality was not significant at 1–2 psu, rising to more than 50% at 3–5 psu (Fig. 5). Thus, sexually mature specimens of *G. fasciatus* tolerate salinities of 4–6 psu, which is the upper limit for this species.

In experiments on stepwise adaptation in *G. fasciatus*, adult amphipods tolerated salinities up to 7–8 psu with 100% survival (Table 2). At salinities of 9–10 psu, their survival decreased to 50–60%. Mortality of 100% was observed at salinities of 11–12 psu. Similar results were obtained with juveniles, whose mortality was in the range 5–25% at salinities of 7 psu and less. After stepwise acclimation in *G. fasciatus*, all females and juveniles tolerated salinity of 7 psu, while males tolerated salinity of 5–6 psu (Table 2). These estimates correspond to the potential salinity tolerance or 'potential euryhalinity' according to Khlebovich (1974) for this species.

We revealed that the reproductive ability of the amphipod *G. fasciatus* significantly decreased at higher

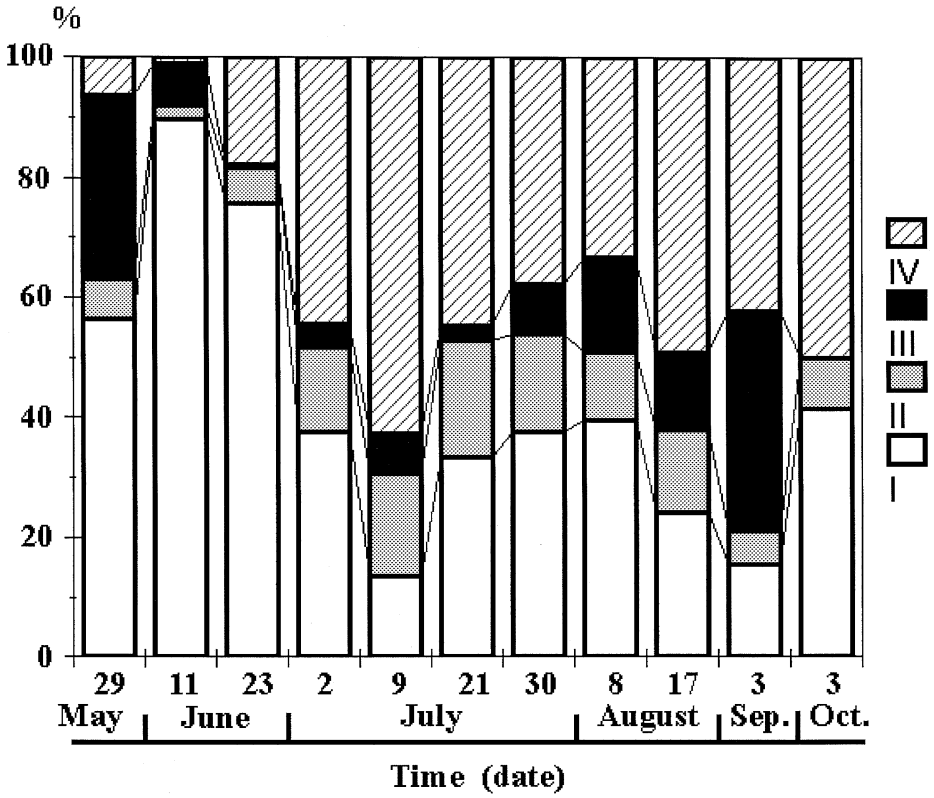

Figure 3. Population structure of *Gmelinoides fasciatus* in the littoral zone of the Neva Estuary in 1998: (I) juveniles, (II) males, (III) females with brood, (IV) females.

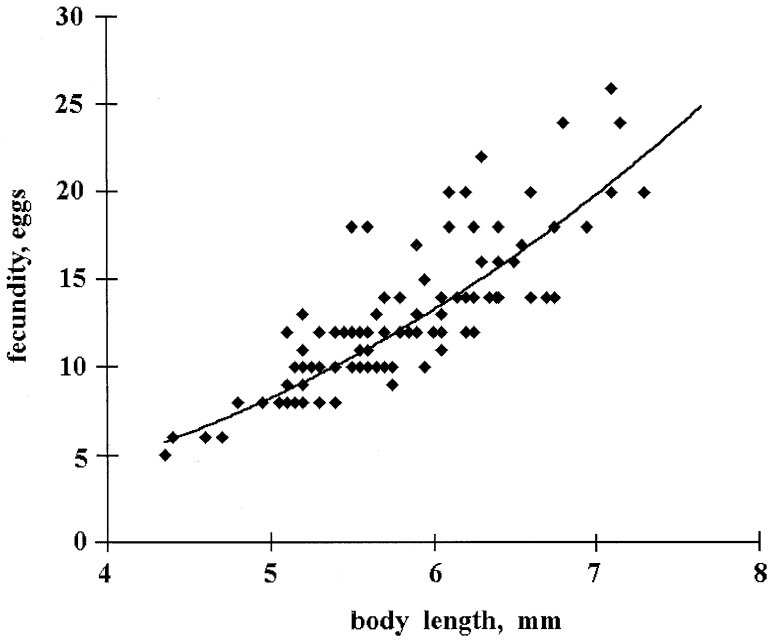

Figure 4. Fecundity (E) in *Gmelinoides fasciatus* females, related to body length (L), in the littoral zone of the Neva Estuary ($E = 0.124\ L^{2.61}$, $R^2 = 0.70$, $n = 120$).

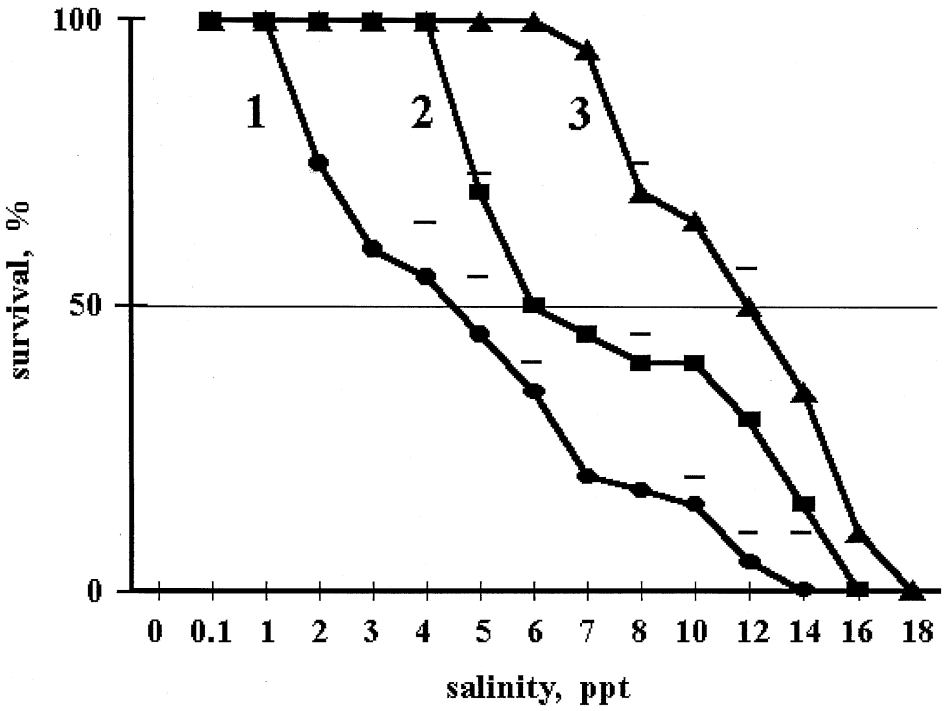

Figure 5. Survival of the amphipod *Gmelinoides fasciatus* after 15 days at various salinities: (1) juveniles, (2) males, (3) females.

Table 2. Salinity tolerance in *Gmelinoides fasciatus*.

Experimental conditions	Upper salinity level, psu	
	Females	Males
Stepwise adaptation (in 1 psu increments at two-day intervals)	7–8	7
Stepwise acclimation (in 1 psu increments at ten-day intervals)	7	5–6

salinities. In freshwater and at 1 and 2 psu, juveniles appeared 11–12 days after fertilization of the eggs. However, in freshwater and at 1 psu, the ratio of hatched juveniles to number of eggs in clutch was 1, while it was only 0.4–0.6 at 2 psu. The juveniles were viable, and their growth and development were normal during the experiment (30 days). At 3–8 psu, females survived for several weeks, but no egg development in their brood pouches was observed. Females either lost their eggs or embryos died at early stages of development.

Experimental studies further showed that the salinity tolerance of *G. fasciatus* depends on water temperature. Survival of amphipods decreased along with increasing temperature (Table 3). At water salinity of 5 psu, the maximum survival in this amphipod (50%) occurred at the lowest experimental temperatures of 12–14 °C. Adult amphipods did not survive in experiments with water temperature of 23–26 °C and salinity of 5 psu. Estimated coefficients of salinity tolerance of *G. fasciatus* decreased with increasing salinity and temperature. This is likely a result of decrease of total adaptive potential of the amphipods during acclimation in brackish waters. At that in water with salinity of 2 psu and 5 psu, the coefficients were higher at low temperatures (12–14 °C) than at medium (16–22 °C) and high (23–26 °C) temperatures (Table 3).

Discussion

Our study shows that at present the invasive amphipod *G. fasciatus* is an abundant species in the studied habitats in the Neva Bay and some locations in the inner Neva Estuary. In the 1980s in littoral communities of the Neva Bay, the amphipod *Gammarus lacustris* Sars was the common and abundant species, with densities of up to 700 ind. m^{-2} in some locations (Panov, 1988). This native amphipod became locally extinct by the period of our study (1998–2000), and we suggest that this was a result of the zebra amphipod invasion.

Table 3. The survival and tolerance to salinity in *Gmelinoides fasciatus* at different salinity-temperature conditions.

Variant	Salinity, psu	Temperature, °C	Survival, %	Coefficient of salinity tolerance
Control	0.2	16.3–22.0	100	1
I	2.0	23.2–25.6	100	0.5 ± 0.05
II	2.0	16.2–21.9	100	0.65 ± 0.05
III	2.0	12.3–14.3	100	0.7 ± 0.1
IV	5.0	23.2–26.0	0	0
V	5.0	16.3–22.1	25	0.5 ± 0.1
VI	5.0	12.1–14.2	50	0.65 ± 0.1

Mechanisms of replacement of the native species by *G. fasciatus* are not clear and require further study. We suppose that two mechanisms are likely. First, adult zebra amphipods are active predators (Bekman, 1962; Panov, 1996) and may prey on juveniles of *G. lacustris*. On the other hand, the invasive species appears more resistant to unfavorable environmental and anthropogenic factors than *G. lacustris*. Perhaps along with it high fecundity and fast population growth, this makes *G. fasciatus* able to successfully replace the native species.

The capacity to tolerate a wide range of salinities is typical for many amphipod species (Kinne, 1970; Khlebovich, 1974). As shown by Bazikalova et al. (1946), *G. fasciatus*, which is a homoiosmotic organism in freshwater, cannot maintain a constant body fluid osmotic pressure at high salinities (i.e. brackish and sea waters) and acquires the features of a poikilosmotic organism. Our experimental results showed that the adult zebra amphipod tolerates brackish water with salinity 5–7 psu, but they are not able to reproduce successfully at salinities higher than 2 psu, because their embryos die. Bazikalova (1945) suggested that *G. fasciatus* is a species of marine origin, because the adult amphipods are able to survive in a wide range of water salinities, and the species is morphologically similar to the Caspian amphipod of the genus *Gmelina*. However, our experimental results showed that development of eggs in *G. fasciatus* was successful only at low salinities, which is in contradiction with the suggestion of a marine origin of this species. These data also indicate that *G. fasciatus* is potentially able to invade parts of the Baltic Sea where salinities do not exceed 5 psu. Because successful reproduction of the species is possible only at water salinities below 2 psu, establishment of permanent populations will be limited to habitats with salinities less than this critical level.

Our experiments also revealed that salinity tolerance in *G. fasciatus* depends on water temperature, with highest tolerance at low temperatures. At a salinity of 5 psu and temperatures of 23–26 °C, the amphipods are not able to survive. Decreasing salinity tolerance with increase in temperature of water has also been found for molluscs (Komendantov et al., 1985). In accordance with Kinne (1964), the tolerance of aquatic animals for salinity decreases at temperatures different from optimum, thus unfavorable salinities are best tolerated at the temperature optimum. We assume that temperatures below 22 °C are more suitable for the acclimatization of zebra amphipod in brackish-water habitats, and this species will not be able to colonize brackish-water areas with high summer temperatures.

The results of the present study indicate that certain salinity and temperature limits exist for zebra amphipod spread in the Baltic Sea. However, considering the ability of amphipods, including *G. fasciatus*, to actively migrate into habitats with favorable environmental conditions (Lindstrom, 1991; Bessolitsyna at al., 2000), the establishment of the zebra amphipod in the brackish coastal habitats of the Baltic Sea, such as the Gulf of Bothnia and Gulf of Riga, is likely in near future.

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Establishment of the zebra mussel, *Dreissena polymorpha* (Pallas), in the Neva Estuary (Gulf of Finland, Baltic Sea): distribution, population structure and possible impact on local unionid bivalves

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Key words: Dreissena polymorpha, establishment, distribution, size structure, unionid bivalves, Gulf of Finland

Abstract

The zebra mussel, *Dreissena polymorpha* (Pallas), has invaded the Neva Estuary (eastern Gulf of Finland) by mid 1980s. In order to assess the current status of the zebra mussel population in the area, the distribution, density and size-frequency structure of *D. polymorpha* were studied in the littoral zone of the Resort District of St. Petersburg in 1998, 2000 and 2001. The results indicate that the species is now established in the inner Neva Estuary. Recruitment of the local *D. polymorpha* population was not regular and occurred only in years with warm summers, while during comparatively cold summers the recruitment was limited or absent (in 1997 and 2000). Even this sporadic and limited recruitment maintained relatively high densities and biomasses of the local *D. polymorpha* population (as high as 2229 ± 801 ind. m^{-2} and 2025 ± 196 g m^{-2}). We consider low availability of accessible substrates and limited recruitment as two possible reasons for the low impact of the zebra mussel on local unionid mussels in the Neva Estuary compared to other recipient ecosystems.

Introduction

During the last two centuries approximately 100 alien species have been recorded in the Baltic Sea, most of which were introduced unintentionally by ship ballast water or hull fouling, or by spreading from primary sites of their deliberate releases into adjacent freshwater bodies (Leppäkoski & Olenin, 2000). The zebra mussel *Dreissena polymorpha* Pallas is one of the first alien species of Ponto-Caspian origin to have invaded the Baltic Sea basin in the beginning of the 19th century as a result of construction of canals in Europe. It was firstly found in the south-eastern Baltic lagoons and estuaries as early as in 1825 (Nowak, 1971; Starobogatov & Andreeva, 1994).

Dreissena polymorpha evolved in estuaries of the relatively warm Paratethys seas and lakes (Starobogatov, 1994). Zebra mussels cannot survive freezing (Karatayev et al., 1998). According to Bij de Vaate (1991), *D. polymorpha* growth starts at 6 °C. Water temperature above 12 °C is required for *D. polymorpha* gamete maturation, spawning and larval production (Garton & Haag, 1993; Kharchenko, 1995; Mackie & Schloesser, 1996; Nichols, 1996). Some authors have suggested that this relatively warm water Ponto-Caspian species is unable to establish populations above 60°N in latitude because of unfavourable temperature conditions (Starobogatov & Andreeva, 1994; Kharchenko, 1995). For more than 150 years, the distribution of the zebra mussel in the Baltic Sea was limited to latitudes below 60°N, and it was not documented in the eastern Gulf of Finland until the 1980s, despite available pathways (waterways from Ponto-Caspian seas to Northwest of Russia) and mechanisms of introduction (shipping). A recent range expansion of *D. polymorpha* in the North of Europe occurred in the 1980s–1990s, when the range of this species has expanded to Irish estuaries of in the North Atlantic (Minchin, 2000), and to relatively cool areas along the northern side of the eastern Gulf of Finland, including the Neva Estuary, the easternmost part of the Baltic Sea (Antzulevich & Lebardin, 1990; Välipakka

et al., 1996; Valovirta & Porkka, 1996; Orlova et al., 1999; Panov et al., 1999).

Dreissena polymorpha is considered an invasive organism, directly or indirectly affecting all levels of aquatic ecosystems (MacIsaac, 1996; Karataev et al., 1997). One of the most negative direct impacts of the zebra mussel in many lakes and rivers is on the diversity of native bivalve mussels, such as unionids (Lewandowsky, 1976; Nalepa et al., 1996; Ricciardi et al., 1998; Burlakova et al., 2000). In some recipient ecosystems, unionids and their shells are often among the most accessible and abundant substrates for settlement of zebra mussel juveniles. Heavy overgrowth of unionid valves by *D. polymorpha* impairs the filter-feeding and movement abilities of the unionids, impedes their growth, and also causes significant deformation of valves, and in some cases leads to extinction of local populations (Schloesser et al., 1996; Karatayev et al., 1997; Ricciardi et al., 1998; Burlakova et al., 2000).

The aim of this study was to assess the current state of the *D. polymorpha* population in the Neva Estuary, including its distribution, density and biomass dynamics in the Resort District of St. Petersburg (inner Neva Estuary) at the northern margin of the zebra mussel range in the Baltic Sea. We also examined whether the zebra mussels infest the local unionids as strongly as they do in other recipient ecosystems. We have focused specifically on the study of age-size structure of *D. polymorpha* settlements, in order to assess whether or not this invasive species has successfully established in the estuary.

Materials and methods

Area description

The Neva Estuary consists of the freshwater area Neva Bay and brackish water parts separated from the Bay by a storm-surge barrier since the early 1980s (Fig. 1). The Neva Bay receives water from the Neva River, the major tributary to the Baltic Sea and a connecting waterway between Lake Ladoga and the Gulf of Finland. The principal area for our investigations was located in the north-eastern part of the Neva Estuary, and covered around 17 km of the shoreline of the Resort District of St. Petersburg (60°09.32′ N–60°11.37′ N; 29°38.34′ E–29°54.89′ E; Fig. 1).

As a result of heavy eutrophication, intensive development of the filamentous green alga *Cladophora glomerata* (L.) Kutz. in the littoral zone is a characteristic feature of the sampling sites (Orlova et al., 1999). High levels of exposure to wave action are also characteristic of the area throughout the vegetation season (from May to October). Heavy storm casts including different bottom organisms are the evidence of disturbance of the environment in the littoral and sublittoral zones by wind and wave actions. The contents of these storm casts depend strongly on wind direction, duration of the storms and water level fluctuations (± 0.5 m). Ice cover is usually present in the area from December to mid April. The thickness of the ice cover in the eastern Gulf of Finland reaches 0.7–0.8 m during cold winters (Davidan & Savtchouk, 1997). Ice abrasion and freezing along with water level oscillations may have a significant impact on littoral zone communities at depths less than 1.5 m during the winter and spring seasons.

Sampling

Quantitative samples of *D. polymorpha* were collected by SCUBA diving along one transect in June 1998 (transect 7; Fig. 1), and along 4 transects in July 2000 and 2001 (transects 7, 11, 13, 15; Fig. 1). Along each transect, samples were taken at standard depths of 0.5, 1.5, 3.0 and 5.0 m with 25 × 25 cm metal frames in three replicates. Sampling along transect 7 was repeated 3 times in the year 2000 (in May, July and September) in order to estimate seasonal variations in size structure of the population, and growth rate of shell length. Environmental variables measured during sampling of *D. polymorpha* included surface and bottom water temperature and conductivity, and oxygen content of the water. Temperature and oxygen content were measured with a WTW Ox 330 oxygen meter. Water conductivity was measured with a Dist WP4 conductivity meter, and then converted to salinity measurements in puss (practical salinity units). SCUBA divers visually estimated the projective cover of bottom by hard substrates at each sampling site.

Quantitative samples were transported alive in plastic bags to the laboratory, where all zebra mussels were detached from substrates, counted and weighed to the nearest 0.01 mg (wet weight with shell). Density (ind. m^{-2}) and biomass (g m^{-2}) of the zebra mussels were expressed as mean ± standard deviation (SD). Data were ^{10}log-transformed (Log(x) + 1) and analysed using a one-way ANOVA implemented with STATISTICA Version 5.0 to estimate the distribution

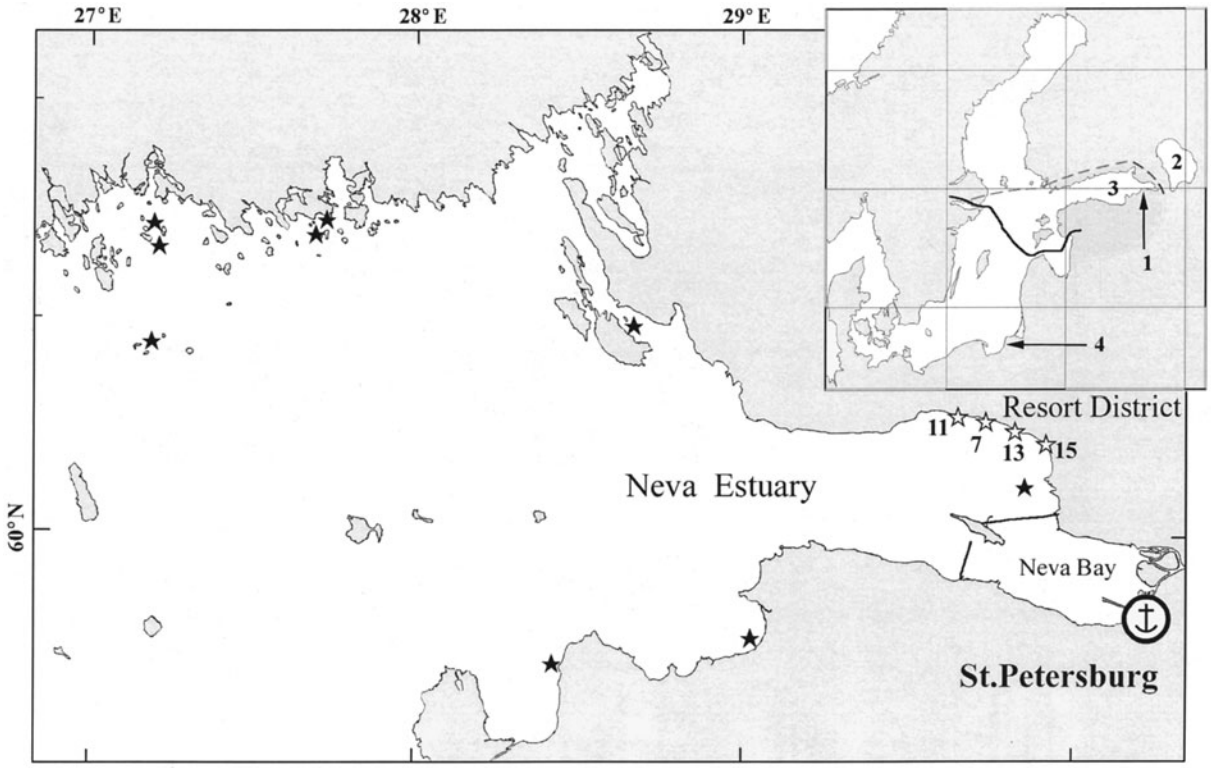

Figure 1. Distribution of *D. polymorpha* in the Neva Estuary and adjacent areas of the eastern Gulf of Finland. Open asterisks with numbers indicate sampling transects in the studied area. Filled asterisks indicate previous records of *D. polymorpha* (after Antzulevich & Lebardin, 1990; Antzulevich & Chivilev, 1992; Valovirta & Porkka, 1996; N. Kovaltchouk, pers. comm.). Areas indicated in the inserted map of the Baltic Sea: 1 = Neva estuary, 2 = Lake Ladoga, 3 = Gulf of Finland, 4 = Vistula Lagoon with contributing Pregel River, the solid line indicates the northern boundary for the *D. polymorpha* range in the Baltic Sea according to Leppäkoski (1984), the dashed line indicates the present northern boundary for the species.

and inter-annual variation of *D. polymorpha* density and biomass.

Shell length (maximum ammonal-posterior dimension) of each *D. polymorpha* individual taken from the quantitative sample was measured to the nearest 0.1 mm using callipers. Using these measurements, the mussels were divided into 1 mm size classes. Size-frequency distribution histograms were used to estimate the size structure of the *D. polymorpha* population. In the year 2000, the size-frequency distribution histograms were effective for visual discrimination of three age-size cohorts [over-wintered (1+), middle-aged (probably 2–3+) and the oldest ages (probably 4–6+)], which were then used to assess the seasonal variation in size structure and growth rate of the shell length. Growth rate of the shell length was calculated as the increase of mean shell length in each of three discernible cohorts and expressed in mm per month.

All collected unionids, both from quantitative sampling and additional ones from qualitative collections, were weighed (wet weight with shell, with the same accuracy as *D. polymorpha*) and the number and biomass of attached zebra mussels were determined. We estimated the extent of unionids infestation by zebra mussels as percent (%) of unionids with *D. polymorpha.* The number of *D. polymorpha* individuals per unionid mollusc (host), biomass of *D. polymorpha* per host and ratio between body mass of host and biomass of overgrowing *Dreissena* served as an estimate of the intensity of unionids' infestation by zebra mussels. Calculated values were compared to values taken from literature.

Results

Environmental factors

During the sampling in May 2000, the average water temperature was 8.7 °C at all stations, both in the

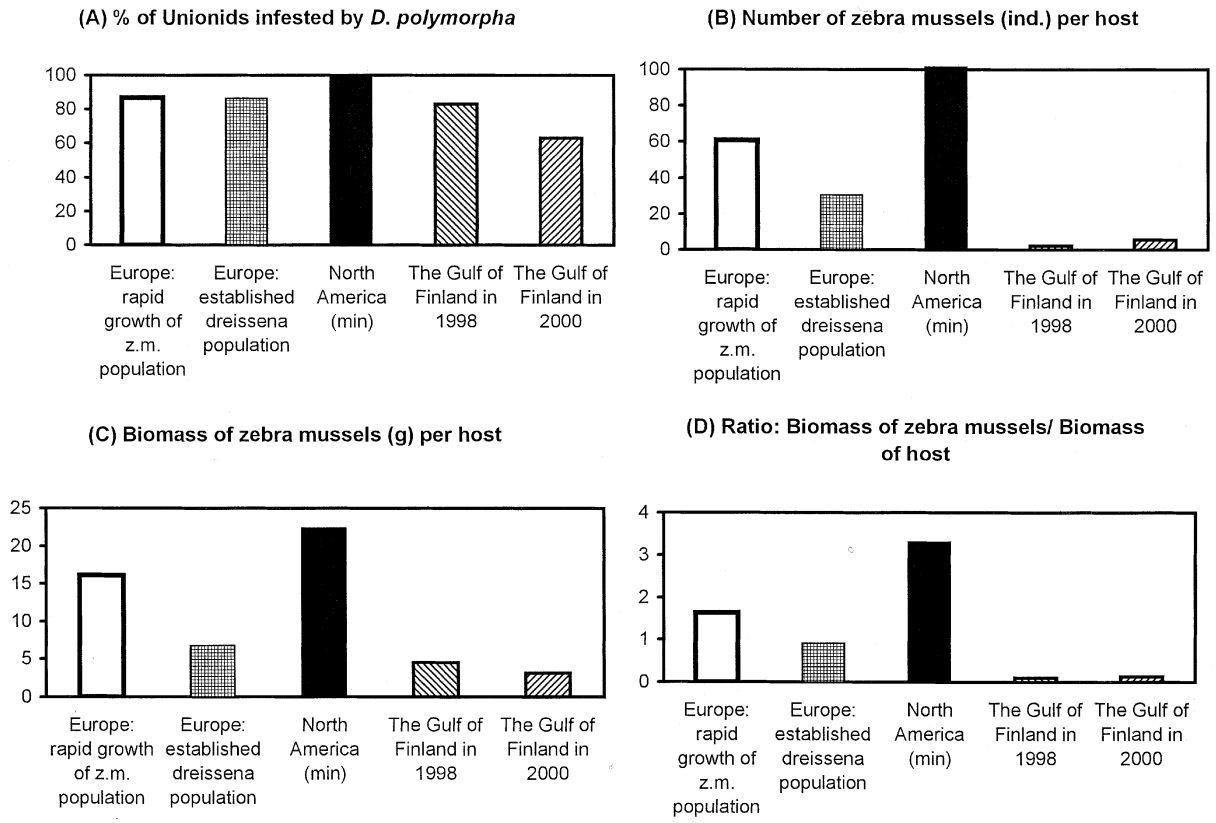

Figure 2. Comparative extent (A) and intensity (B, C, D) of infestation of unionids by *D. polymorpha* in the Neva Estuary and in other recipient areas in Europe and North America, based on our observations in 1998 and 2000 and the review by Karatayev et al. (1997). Only minimal values (Lake Vineyard) were taken for North America.

surface and bottom layers. In July 2000, water temperature ranged from 18.6 to 21 °C in the surface water and from 17.4 to 18.1 °C at the bottom. In September 2000, water temperature had decreased to 12–14 °C. In July 2001, surface water temperature ranged from 21.8 to 25.5 °C, and the upper bottom temperature ranged from 21.5 to 23.6 °C along transects, with the highest temperatures at the most shallow sampling sites. The deep-water areas of the Neva Estuary and eastern Gulf of Finland (depths >15 m) are stratified during summer and sometimes vertical temperature gradients, with a temperature decline of 2.5 °C per m of depth profile, occur (Panov et al., 1999). Oxygen saturation during daytime at depths from 1.5 to 5.0 m always exceeded 90% for the whole period of observations. Salinity measured from May to September in 1998–2001 ranged from freshwater (<0.4 psu) to brackish water (around 2 psu).

Colonization of hard substrates and infestation of unionids by D. polymorpha

Hard substrates were available at all depths along transect 7, with the projective cover ranging from 23 to 60%. In transects 13 and 15, hard substrates were present at the depths from 0.5 to 3.0 m, ranging from 57 to 100%. At transect 11, hard substrates were present only at the depths 0.5 and 1.5 m (62 and 75%, respectively).

In 9 frames (100 × 100 cm), collected on 29 June 1998 at depths 1.5, 3 and 5 m along transect 7, the substrate consisted of stones and debris (91%) and, to a lesser extent, of live molluscs, both zebra mussels (5%) and native unionids (4%). Substrates of all types and sizes were colonized by *D. polymorpha*. 87% of the total number of zebra mussels had settled on stones and debris, while 11% were found attached to large individuals of the same species and only 2% were found on unionids. These results indicate that shells of unionid mussels were not a preferred type of substrate

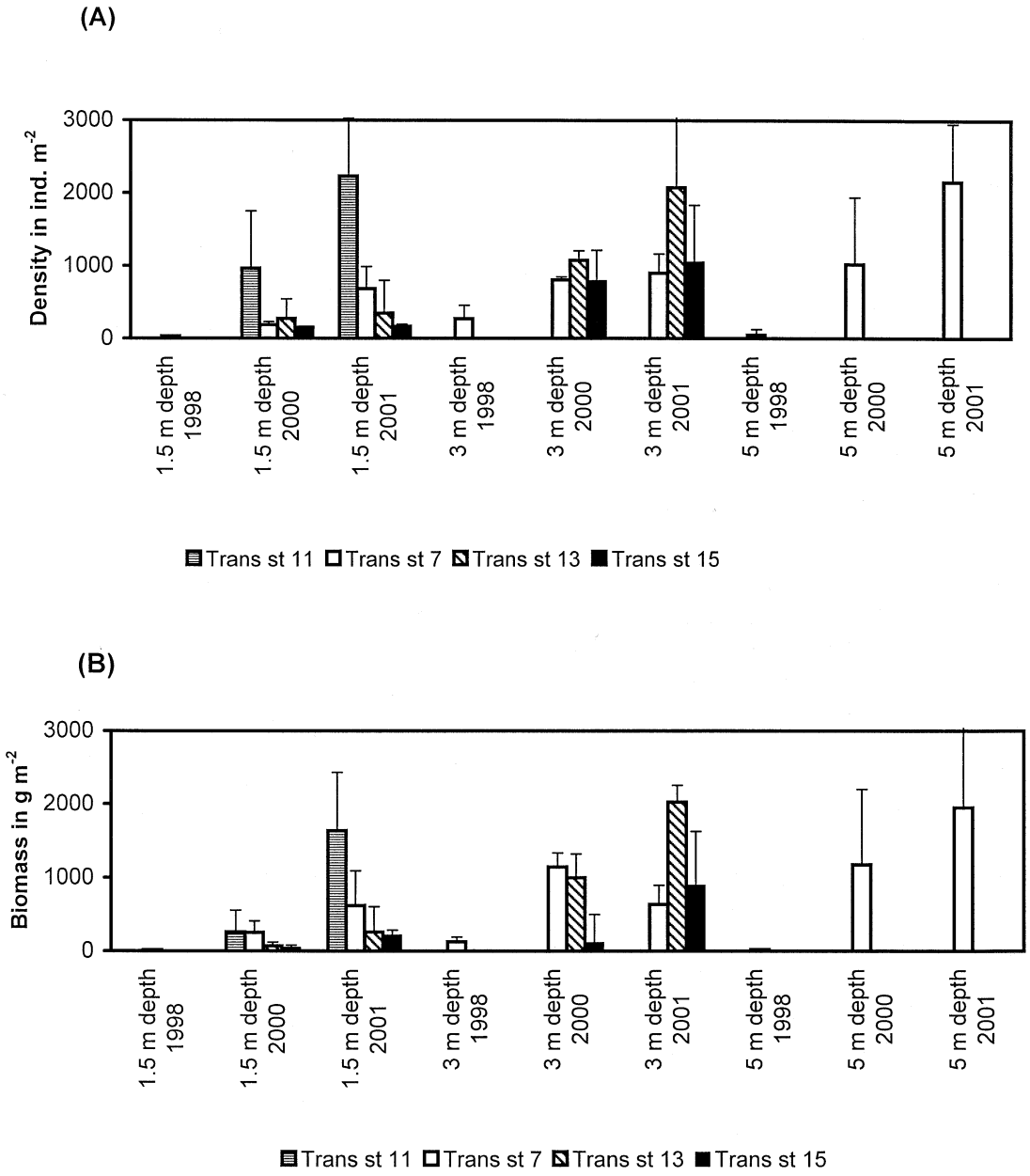

Figure 3. Quantitative characteristics of the *D. polymorpha* population from the Neva Estuary along four transects in June 1998, July 2000 and July 2001.

for zebra mussels than shells of larger conspecific individuals. In 1998 and 2000, around 60-70% of all collected unionids were colonized by *D. polymorpha*, as observed in other recipient aquatic ecosystems in Europe (Fig. 2A). However, the intensity of infestation was much lower than found in other water bodies (Fig. 2B, C, D).

Spatial and temporal variations of D. polymorpha *abundance and biomass*

Variations in density and biomass of *D. polymorpha* were related to the locations and depths of the transects (Fig. 3, Table 1). No permanent settlements of *D. polymorpha* were found at sites less than 1–1.5 m deep where ice abrasion, freezing and water level oscillation

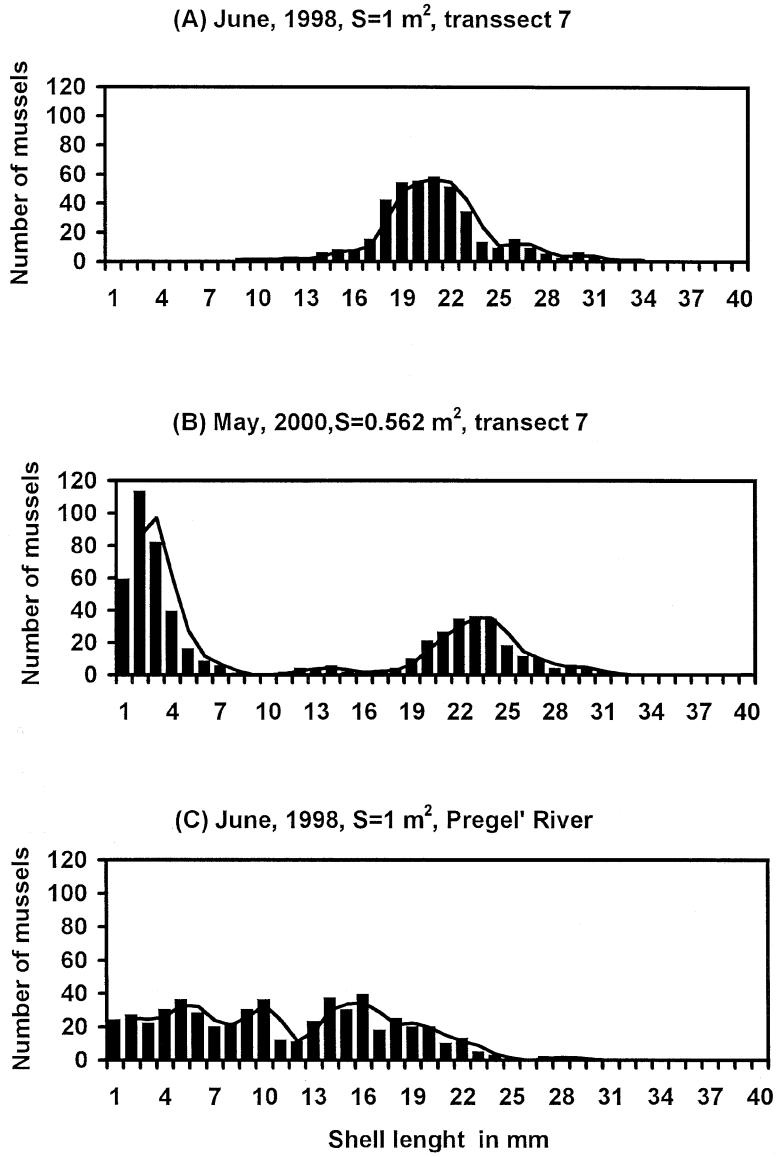

Figure 4. Size structure of the *D. polymorpha* population from the Neva Estuary, eastern Gulf of Finalnd (A, B) and of the *D. polymorpha* population from the Pregel River, Vistula Lagoon (C).

may affect the living assemblages. High abundance and biomass of the species in the study sites were usually found at depths of 3 to 5 m in the central transects 7 and 13, except for transect 11 where high abundance of zebra mussels was found at a depth of 1.5 m (Fig. 3). Variations in density and biomass of *D. polymorpha* between July 2000 and July 2001 were not statistically significant (Fig. 3, Table 1). However, both were significantly larger in 2000 and 2001 (2229 ind. m^{-2} and 2025 g m^{-2}) relative to 1998, when mean values did not exceed 125 ind. m^{-2} and 269 g m^{-2}.

Size structure of the D. polymorpha *population and growth of shell length*

In June 1998, young molluscs were not found in the local *D. polymorpha* population (Fig. 4A). However, a large cohort of over-wintered young mussels settled in autumn of the previous year was found in May 2000 (Fig. 4B). We used samples from the Pregel River (Vistula lagoon), collected in June 1998 (area 4 in the inserted map of the Baltic Sea in Fig. 1) for comparative analyses of size structure of two Baltic Sea

Table 1. One-way ANOVA results for *D. polymorpha* density and biomass variation. Data from samples collected in July 2000 and July 2001 along transects 11, 7, 13 and 15 at water depths 1.5–5 m were used. * = Data from samples collected in June 1998, July 2000 and July 2001 along transect 7 only, df = degrees of freedom.

Variable	Density			Biomass		
	df	*F*-value	*p*-value	df	*F*-value	*p*-value
Spatial variation:						
Transect localization	3.68	6.579	<0.001	3.68	8.994	<0.000
Depth	2.69	17.554	<0.000	2.69	12.443	<0.000
Temporal variation:						
Year	1.70	0.248	<0.620	1.70	0.794	<0.376
Year*	2.24	30.691	<0.000	2.24	1.134	<0.000

populations of the zebra mussel. The size structure of the Pregel River population (Fig. 4C) was regular with the presence of all size classes. This age-size frequency pattern reflects the regular annual recruitment in Pregel River population of *D. polymorpha*, in contrast to the Neva Estuary population.

In the Neva Estuary the youngest cohort (shell length 1–8 mm), found in May 2000, persisted through the whole season as well as two older cohorts (Fig. 5). Limited recruitment in the *D. polymorpha* population was observed during summer 2000, which resulted in the appearance of only few 0+ individuals of zebra mussels in our samples in September (Fig. 5, lower chart). The analysis of the population size structure in July 2001 at depths of 1.5 m along the study area has revealed the presence of this cohort at all localities (Fig. 6). The highest abundance of these one-year old mussels was found in transect 11 (Fig. 6A), which was the sampling site furthest away from the freshwater Neva Bay (Fig. 1). The same was observed for the two-year old cohort. At 1.5 m of depth, the total density and biomass of the zebra mussels in the outer part of the estuary (transect 11) was higher than in the inner part (transects 13 and 15) (Fig. 3).

Seasonal changes in the size structure of *D. polymorpha* in 2000 allowed the estimation of growth rates in three cohorts. Individuals of the 1+ cohort were the fastest growing. Mean shell length in this cohort increased from 3 mm on 17 May to 8 mm on 9 July, and had reached 13 mm by 12 September 2000 (Fig. 5). The monthly rate of linear growth in this cohort averaged 2.5 mm month^{-1} throughout the time of observation. The largest, probably 4–6-years old individuals with shell length of 17–31 mm in May, had the lowest monthly rate of linear growth, averaging 1 mm month^{-1} from May to September. The middle-aged cohort was represented by few individuals of likely 2–3 years old zebra mussels, whose shell length varied at the beginning of observation from 11 to 19 mm. The monthly rate of growth for this cohort was estimated at less than 2 mm month^{-1}.

Discussion

Our sampling survey revealed dense permanent settlements of *D. polymorpha* at the majority of the studied sites along the coastal zone of the Neva Estuary. The establishment success of this species is evidently facilitated by relatively favourable environmental conditions in the coastal zone. Summer temperatures in the Neva Estuary coastal zone in 2000 and 2001 were sufficient for *D. polymorpha* growth and above the limiting temperatures for reproduction. Estimates of salinity in the Neva Estuary littoral zone in 1998–2001 corresponded to favourable conditions for the species (Strayer & Smith, 1993) and oxygen saturation of waters in the studied area was also sufficient for oxyphylic species as *D. polymorpha* (Karatayev et al., 1998). As a result, the density and biomass of the zebra mussel along the study area in the Neva Estuary was close to the values indicated for lower reaches and estuaries of other eastern European rivers at localities without perennial macrophyte vegetation (1000 ind. m^{-2} and 185 g m^{-2} in lower Danube River, and 1730 ind. m^{-2} and 1240 g m^{-2} in the South Bug Liman; Karatayev et al., 1998).

Availability of hard substrate for attachment of *D. polymorpha* is one of the major factors affecting its distribution (Karatayev et al., 1998). Hard

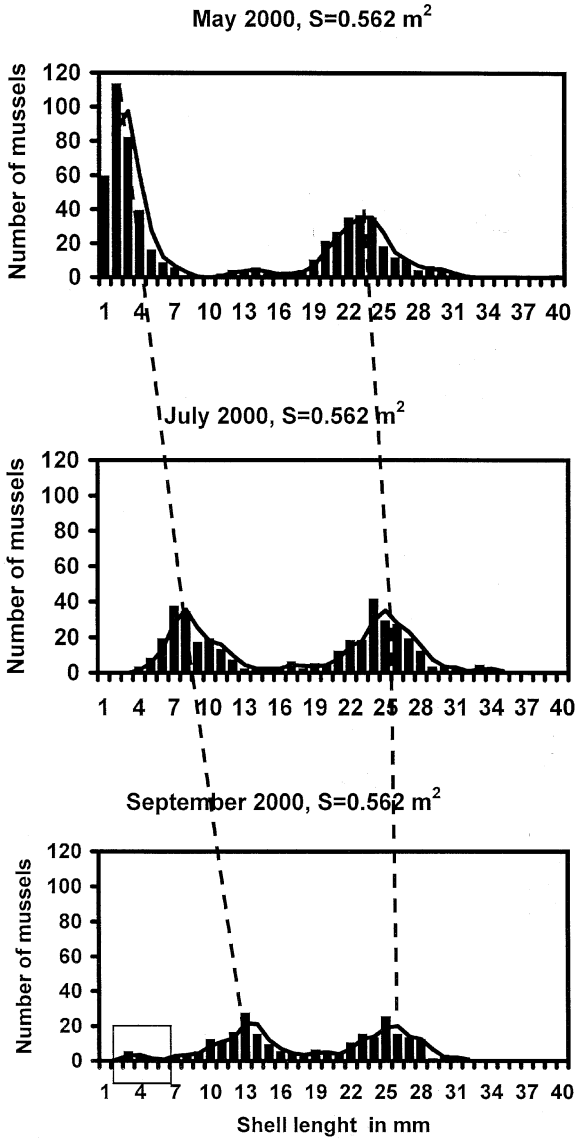

Figure 5. Size structure of the *D. polymorpha* population in transect 7 throughout the warm season of the year 2000. Black dashed lines indicate changes in mean size of two (youngest and oldest) cohorts. The quadrates below the picture indicate the new 0+ cohort, consisting of juveniles settled in late summer 2000. Each of the histograms is based on all nine quantitative samples collected at depths 1.5, 3 and 5 m.

substrates in the Neva Estuary were available for colonization by *D. polymorpha* at all locations studied. Probably, the availability of various suitable hard substrata for the settlement of the zebra mussel in the studied area of the Neva Estuary has resulted in a lower level of infestation of the local unionid mussels by *D. polymorpha*. Despite the fact that the number of infested unionids (60–70%) was as high as in other European ecosystems (Fig. 2A), the intensity of infestation, characterised by all three estimated parameters (Figs 2B, C, D) was comparatively low. The low number and biomass of *D. polymorpha* per host is unlikely to cause any damage to shells and has no serious impact on the behaviour of unionids in the studied area, unlike the situation in Byelorussian lakes (Burlakova et al., 2000), North American Great Lakes (Nalepa et al., 1996; Schloesser et al., 1996; Karatayev et al., 1997) and Mississippi River (Ricciardi et al., 1998), where some species of Unionidae have become extinct after establishment of this invasive species. Insufficient recruitment of *D. polymorpha* in years with cold summers is probably another important reason of relatively low intensity of local unionids infestation by zebra mussels in the Neva Estuary.

The distribution of *D. polymorpha* toward the shoreline in the Neva Estuary coastal zone is probably limited by water level fluctuations and ice abrasion and freezing effects, like in other ecosystems in Europe (Karatayev et al., 1998). In late summer – early autumn, intensive developments of filamentous algae (*Cladophora glomerata*) may also be a limiting factor for the permanent settlement of zebra mussels. During this period, the biomass of filamentous algae in shallow locations (depths 0.5–1.0 m) may reach up to 500 g dry weight m^{-2} (Orlova et al., 1999). Settlement of zebra mussel juveniles on such unstable substrate as filamentous algae would result in removal of settled juveniles from the population after algal detachment or die off. This effect might be also responsible for the lower density and lower biomass at shallow depths (1–1.5 m) in comparison to deeper locations.

Dispersal of *D. polymorpha* to the deeper, central part of the Neva Estuary is most likely limited by both a lack of hard substrate and low temperature of near-bottom waters, which is significantly below that required for growth and reproduction in *D. polymorpha*. The trend of increasing density and biomass of *D. polymorpha* toward the lower reaches of the estuary (from transect 15 to transect 11) may result from a prevailing passive drift of the zebra mussel larvae up west with the near shore current induced by Neva River water discharge, and their subsequent settlement downstream the parental population. As it was observed in the Volga River reservoirs (Kirpichenko, 1997) and in the Illinois and Hudson Rivers (Padilla et al., 2002), *D. polymorpha* larvae in flowing habitats, such as reservoirs and large rivers (and evidently estuaries of large rives, e.g. the Neva River), can settle

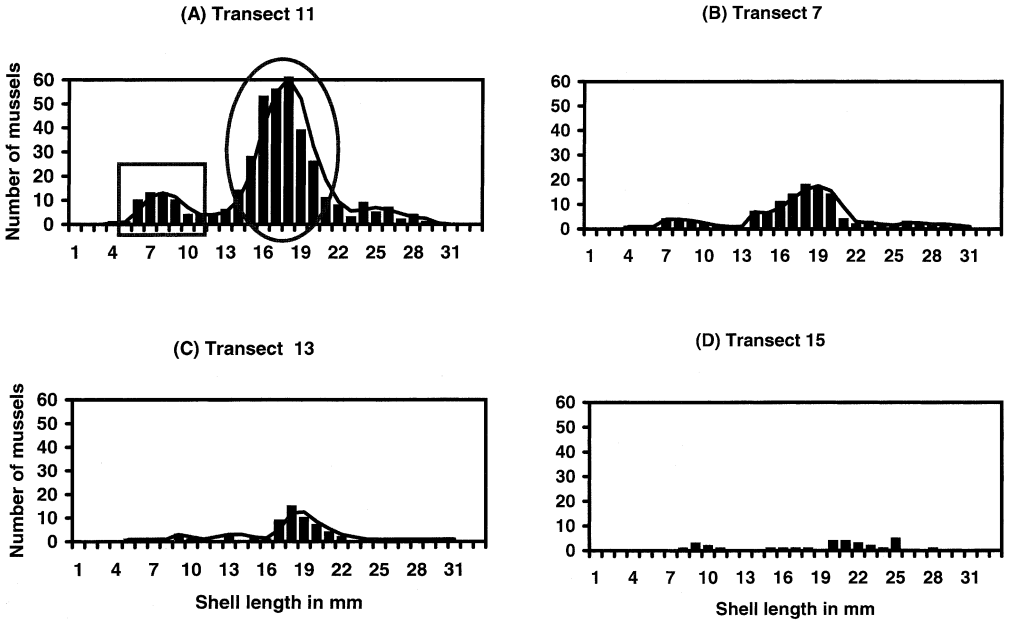

Figure 6. Size-frequency distribution of the *D. polymorpha* population in July 2001 at depths 1.5 m along four transects. Quadrates indicate the 1+ cohort (molluscs settled in 2000); circles indicate the 2+ cohort (mulluscs settled in 1999).

far downstream from the locality where they were produced.

Temporal variations in density and biomass of *D. polymorpha* in the Neva Estuary are most likely due to the success of recruitment. The observed rapid increase of the zebra mussel density and biomass in 2000–2001 can be explained as a result of successful recruitment in summer 1999. The size structure of the Neva Estuary population, located at the northern margin of the species's range in the Baltic Sea, demonstrates a lack of breeding in summer 1997, or absence of sufficient passive drift of larvae from adjacent localities in the estuary, as shown by our survey in 1998. Valovirta & Porkka (1996) studied a population of *D. polymorpha*, located in Finnish waters along the north coast of the eastern Gulf of Finland and concluded that '... the species can reproduce in the north coast only in very favourable years'. The appearance of a new large cohort of over-wintering young individuals in the studied area (Neva Estuary) in May 2000, resulting from settlement during late summer-early autumn of the comparatively warm 1999 season, shows that successful recruitment of *D. polymorpha* is possible in locations north of latitude 60°N. During such successful seasons, occurring perhaps every 2–3 years, the population is able to restore its high level of density and biomass. In contrast, during periods with limited or absent recruitment, the abundance of zebra mussels in the estuary may decline. Despite strong inter-annual fluctuations in density and size structure of the *D. polymorpha* population, the population has persisted in the Neva Estuary for at least 15 years (Antzulevich & Lebardin, 1990).

We suggest two main reasons for the observed irregular recruitment of *D. polymorpha* in the Neva Estuary. Firstly, temperature is obviously an important limiting factor for successful reproduction during cold summers according to temperature limits provided by literature. Secondly, the hydrological features of the studied area, which is highly dynamic in terms of currents and wind exposure, may be an important limiting factor for successful settlement and attachment of the mussel's juveniles, as suggested by the gradient in distribution of abundance of *D. polymorpha* (Figs 3, 6) along the coast at shallow water sites. The passive drift with near shore currents can remove a large part of zebra mussel larvae from the study sites even in warm years with successful reproduction. These larvae may settle along the shoreline westward. The population of *D. polymorpha* encountering even more hostile conditions along the south coast of Finland (Valovirta & Porkka, 1996) might have been established as a result of this passive drift of larvae, originating from the studied localities in the Neva Estuary. Other pools of larvae may settle in deeper parts of the Neva Estuary with unfavourable temperature conditions and lack of

suitable bottom substrates. The larvae also may drift to the parts of the Gulf of Finland with the water salinities exceeding 5.5 psu. According to Valovirta & Porkka (1996), 5.5 psu is the upper salinity limit of zebra mussel dispersal in the Gulf of Finland.

Despite that temperature of water in the studied areas of the Neva Estuary is not always optimal for recruitment, it is sufficient for the mussel growth. The rates of shell length growth in the studied population did not differ significantly from the estimates for other European populations (Walz, 1978).

The results of our field surveys in 1998–2001 indicate that the population of the invasive Ponto-Caspian mussel *D. polymorpha* in the Neva Estuary can be considered as established and permanent. Irregular recruitment is a particular characteristic of the Neva Estuary zebra mussel population. More research on metapopulation dynamics along the whole system of the Neva Estuary, including plankton larval stages and breeding strategy is required to discover the causes of these observations. The infestation of local bivalves by the zebra mussel in the Neva Estuary is less intensive than in other studies. This may be explained both by availability of other hard substrates for colonization by settling juveniles and by non-regular recruitment of the *D. polymorpha* population.

Acknowledgements

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The round goby (*Neogobius melanostomus*) in the Gulf of Gdańsk – a species introduction into the Baltic Sea

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Key words: non-indigenous species, ballast waters, shallow waters, Baltic

Abstract

In recent years, information concerning the awareness of organisms accidentally introduced into the Baltic Sea has substantially improved. Non-indigenous Estuarine and Marine Organisms (NEMO's) are hazardous for the Baltic ecosystem. Currently, about one hundred species are identified as accidentally or intentionally introduced into the Baltic Sea. Ballast waters and escape from aquaculture are the most important invasion vectors. During the last decade, an invasion of the round goby (*Neogobius melanostomus*) – a Ponto-Caspian fish species has been observed in the Gulf of Gdańsk. The first record of this fish in the Baltic Sea is from 1990. Early detection of the invader enabled the study of population growth and changes in the area. The first years of invasion were characterized by low numbers of individuals and a limited distribution. Later, the round goby gradually colonized all shallow waters in the western part of the Gulf of Gdańsk. Initially the fish inhabited stony and rocky habitats, but later it also occupied sandy bottoms. The round goby is now the dominating fish species in most of the shallow waters of the Gulf of Gdańsk. Two main factors account for the successful invasion of this fish in the region: the state of ecosystem at the time of the invasion and the biological features of *N. melanostomus*. In the late 1980s, the shallow waters of the Gulf of Gdańsk were almost devoid of piscivorous fishes. Concurrently, bivalves (a preferred prey of the round goby) have increased. Important is also parental care of laid eggs and reproductive strategy. Population growth potential enables the colonization of nearby regions. The first round gobies in the Vistula Lagoon were collected in 1999 and colonization of other Baltic Sea areas is anticipated.

Introduction

Human society changes natural environments often without previous detailed elaboration of possible consequences. Industrial progress involving direct changes in nature often modifies general ecological equilibria. For example, a growing marine traffic during the last century has resulted in an increase of unintentional species introductions. New species can change the energy flow through the ecosystem, its food web and its ecological balance. Introduced species can remove or at least alter the occurrence of native species in their habitats (Elton, 1958; Lodge, 1993; Ricciardi & MacIsaac, 2000).

Currently, about one hundred known species have been introduced into the Baltic Sea by humans. Among them are organisms living in soft-bottom and littoral habitats and those occupying the pelagic zone; there are a few parasites, as well (Leppäkoski & Olenin, 2000a, b). Taxonomically introduced species belong to different taxa. There are plants (algae and vascular plants), coelenterates, molluscs, annelids, crustaceans, fish and some species belonging to other taxonomic groups. There are 27 fish species introduced into the Baltic Sea, but only the round goby is without doubt introduced by shipping (Leppäkoski & Olenin, 2000b). Introduced species live in diverse habitats. Some of them are common throughout the Baltic Sea, whereas others only occur in specific regions. Each introduction is different and each species has its own biological features promoting invasion. Although many foreign species are introduced deliberately into the Baltic Sea every year, only a small

proportion of them are successful in becoming established. New species in the Baltic Sea originate from several geographical regions. Most species invade from the North America (30%) and the Ponto-Caspian region (30%). Other introduced species originate from south-eastern Asia, the Indo-Pacific region and from the Pacific Ocean. New species are mainly introduced by shipping (predominately in ballast waters), stocked intentionally or released in association with aquaculture.

The invasion of the round goby (*Neogobius melanostomus*) from the Ponto-Caspian region is a case of new species introduction into the Baltic Sea. The round gobies invaded the Gulf of Gdańsk in the end of the 1980s. This paper addresses several questions concerning the round goby invasion. Is it an unusual case or is it a sign of a general trend in the Baltic ecosystem? Why has the round goby succeeded to colonization new regions so well? Does the round goby compete with native species? What will the future geographical range and abundance of the round goby in the Baltic Sea be?

Material and methods

This paper presents selected results of investigations carried out during the last ten years on the invasion of the round goby in the Gulf of Gdańsk. The areas occupied by the round goby during its invasion are presented based on collected data on the occurrence of this species. The data are derived from different sources: control sampling (passive nets and trawls), underwater observations and information from fishermen. Beam trawls (2 m wide) were used to collect data on the fish abundance in deeper sandy regions (5 to 50 m of depth). Trawling took place in the first half of July in each year of investigation. Fish length was measured as the total body length and fish weight as the total wet weight of the body. The fish age was determined by otholits (sagitta) readings.

Results

In 1990, the first round gobies were caught in the Gulf of Gdańsk (Skóra & Stolarski 1993) as the first sign of a starting invasion. These fish were adults (about three years old), which suggests that the first successful introduction had taken place some years earlier. The invaded region is distinctly separated from any other place of round goby occurrence (Berg, 1949; Miller, 1986; Charlebois et al., 1997).

Transport in ballast waters was, most probably, the mechanism that distributed the round goby from its native regions over long distances. Despite morphological and genetic investigations (Dougherty et al., 1996; Dillon & Stepien, 2001), the origin of round goby introduced into the Gulf of Gdańsk is still unknown. It is also unknown which route the vessels used to transport the round goby to the Gulf of Gdańsk. Theoretically, there are three possible ways (Fig. 1): (1) the shortest route, from the Black Sea through the Dnepr, Prypec, Pina, Bug and Vistula, (2) a longer route, mainly through freshwaters, from the Sea of Azov (through Don) or the Caspian Sea and then through the Volga, Rybinski Reservoir, Moscow River, Onega Lake and the Gulf of Finland, (3) the less probable, but still possible, route around Europe, from the Sea of Marmara, through the Mediterranean, the Atlantic Ocean and the North Sea. The round goby was observed in Moscow River near Moscow City in the mid 1980s (Sokolov et al., 1989). This may suggest that the route through Volga was the most probable. The invasion of the round goby into the Gulf of Gdańsk and into the Laurentian Great Lakes (Jude et al., 1992, Crossman et al., 1992) occurred at about the same time. Therefore, it is also possible that the round gobies came to the Gulf of Gdańsk from North America with ship ballast water. However, genetic analyses (Dillon & Stepien, 2001) have rejected this hypothesis.

The first round gobies were caught near the port in Hel, on the tip of the Hel Peninsula (Fig. 2) in June 1990. Later in the same year, more round gobies were caught near the harbours of Hel and Gdynia. During the next two years, there were no changes in the area occupied by the round goby and population growth was slow. This was followed by a large increase in numbers and colonization of Puck Bay. In 1994, the round goby was present in all shallow waters of Puck Bay and some individuals were sampled in deeper open waters at about 40 m of depth. In following years, the round goby colonized areas to the south and east of the original area, reaching the main outlet of the Vistula River and the western border of the Gulf of Gdańsk in 1997. During the following two years population growth was observed in the previously colonized regions, but no new regions were colonized. In 1999, the round goby was one of the dominant species in shallow water zone of the western part of the Gulf of Gdańsk.

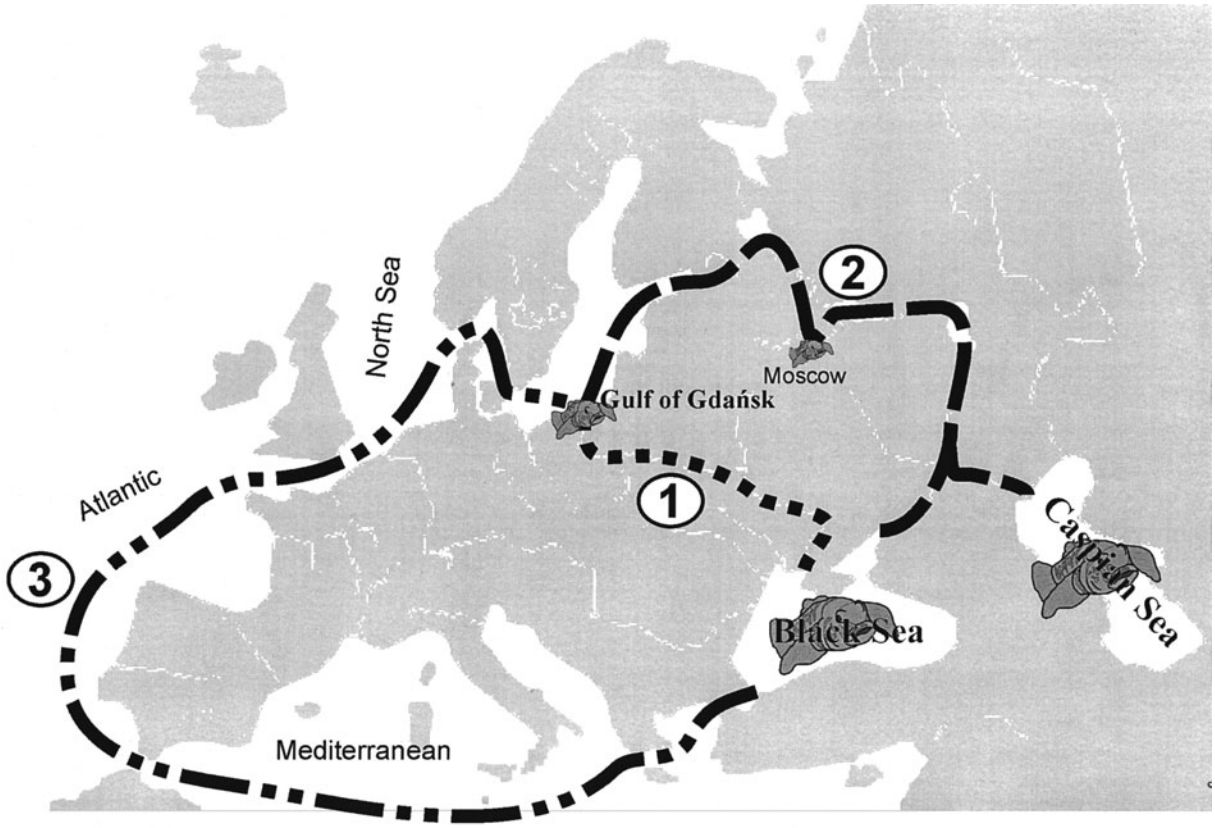

Figure 1. Theoretical ways of round goby introduction into the Gulf of Gdansk.

The round goby has now started to invade the Vistula tributary. In 2001, round gobies were observed 40 km up from the Vistula outlet. Simultaneously, increases in the abundance of the round goby in previously colonized regions occurred. Observed abundances in preferred localities varies from a few ind. m^{-2} to (in a few limited areas) $\sim$30 ind. m^{-2}. Currently, three geographically separate groups of round gobies exist in the Gulf of Gdańsk and its vicinity (Fig. 2). The stable and well-established group that occupies shallow waters (down to 20 m of depth) in the western part of the Gulf of Gdańsk has the highest abundances. The second group, on the western border of the Gulf of Gdańsk, has low abundances and is not increasing at a fast rate. The third group, in the Vistula Lagoon, started to increase rapidly in abundance in 2001. There are no morphological or genetic differences between the groups. This suggests that new groups originate from the first established group inhabiting the western part of the Gulf of Gdańsk. Discrete distribution analysis suggests that ballast waters was the way new groups were established.

Discussion

The round goby has colonized the Gulf of Gdańsk. Why was its invasion so successful? What characteristics of the species promoted population growth? Based on observations of round gobies in the Gulf of Gdańsk some features can be identified. Round gobies typically prefer shallow waters (from $<$1 m to about 20 m of depth) and they always occur near bottom. They are seldom found in deeper waters. Hard bottoms are preferred by round gobies (Berg, 1949; Miller, 1986; Charlebois et al., 1997). The invasion of the sandy bottoms of the Gulf of Gdańsk has started from the harbour regions where the concrete piers acted as refuges (Skóra & Stolarski, 1993).

In the Gulf of Gdańsk, the round goby grows up to 25 cm in total length. Mostly, individuals from 8 to 18 cm in length are found. Twenty-five cm is one of the largest sizes recorded for this species in the world (Berg, 1949; Charlebois et al., 1997). The round goby is the largest or one of the largest fish in sampling area and provides the privilege of almost undisturbed popu-

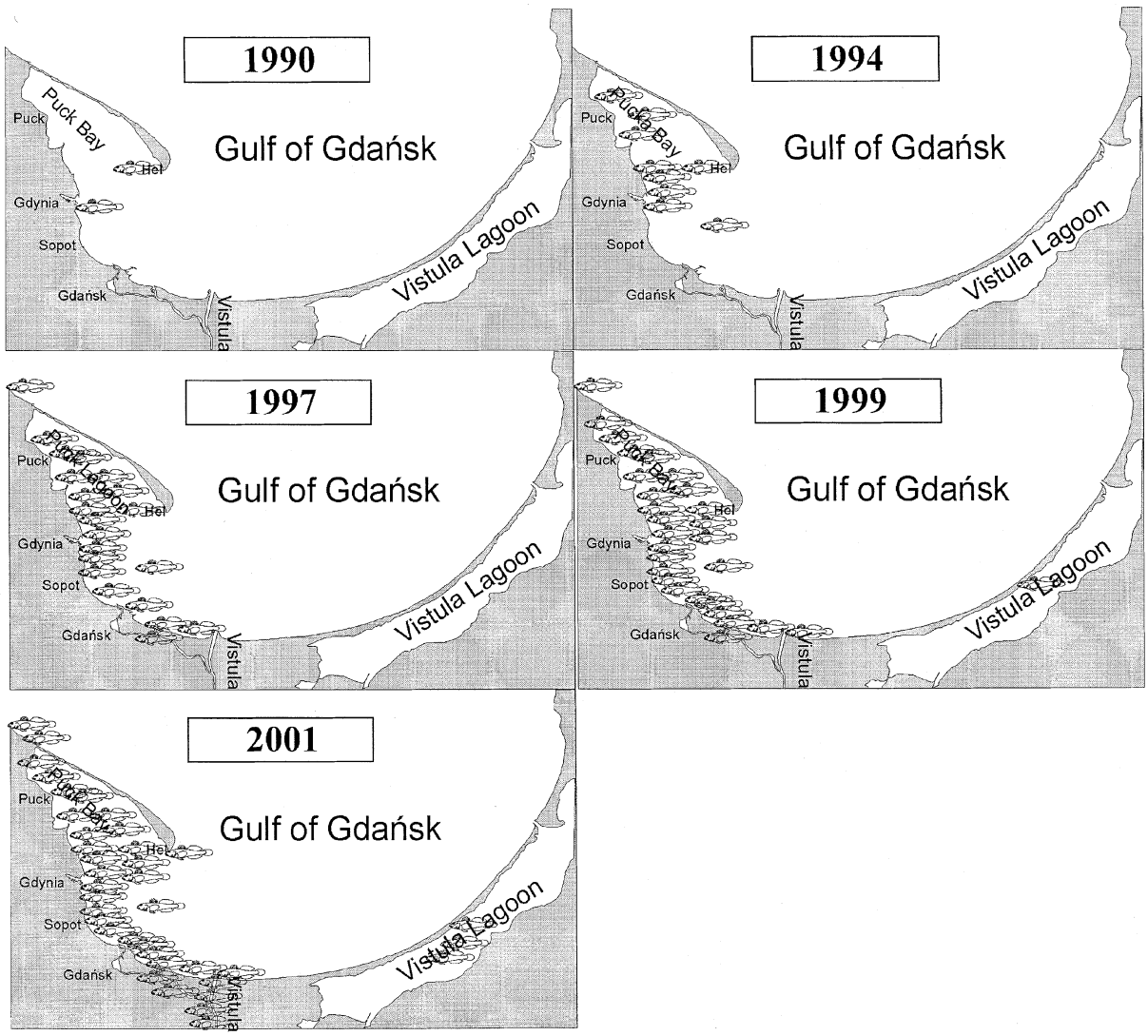

Figure 2. Sequence of the round goby invasion in the Gulf of Gdansk.

lation growth. The life span of the round goby is rather short, three to four years. Generally, females live about three years. Males can live one year longer and they attain larger sizes (Berg, 1949; Nikolski, 1954). In the Gulf of Gdańsk, spawning of the round goby takes place from the beginning of May to the end of September, prolonging throughout the whole warm part of the year. Males to females ratio is three or two to one (taking into account only mature individuals despite method of sampling), which is contrary to native populations where the sex proportion is more or less equal (Kovtun, 1979). This overproduction of males maybe connected with invasion strategy, males being more vulnerable to predation due to their guarding of the nests.

Round gobies are multi-spawners (Kovtun, 1977). In the Gulf of Gdańsk, the round goby females may exhibit 2 to 4 separate breeding events. This increases the opportunity, for at least a part of next generation, to grow in optimal environmental conditions. Round gobies lay their eggs in nests guarded by males. More than one female can lay eggs in one nest (Moskalkova, 1996) and eggs in different stages of development can be found in one nest. The nest must be a solid substrate, and all solid elements on the bottom can be used. For example stones, rocks, parts of wood, roots of vascular plants or even dumped wastes can be util-

ized by round goby. In the Gulf of Gdańsk, where suitable nesting sites are limited, the distance between nests is much shorter than in other regions. Sometimes the distance between one nest border to the other is shorter than 1 cm.

Round gobies can live in marine, brackish and freshwater environments (Charlebois et al., 1997). The salinity in the Gulf of Gdańsk is about 7 practical salinity units (psu). Hypothetically, round gobies can live in full oceanic salinity, but no such population exists. The range of round goby migration is short, mostly within some 100-m distance. The longest migrations (up to 10 km) take place in late autumn and early spring, when round gobies migrate to and from deeper waters (Berg, 1949; Miller, 1986).

Round gobies are typical benthic feeders (Berg, 1949; Miller, 1986; Skazkina & Kostyuchenko, 1968; Kovtun et al., 1974). Bivalves (mainly blue mussel) constitute about 60% of the round goby diet in the Gulf of Gdańsk. Arthropods (mainly *Idotea*) play an important role in the diet of juveniles (Skóra & Rzeźnik, 2001).

In the Gulf of Gdańsk, round gobies are retrieved in commercial fishery rates as by-catch. The large population size suggests that commercial exploitation would be feasible but markets are lacking. Round goby could be prey for piscivorous fish (predators), but in the altered shallow water ecosystem of the Gulf of Gdańsk, there are almost no fish predators left (Skóra, 1993). Instead, the round goby is an important food source for cormorants constituting at least about 60% of the diet (Bzoma, 1998). From time to time, also young cod feed on round goby in the shallow waters of the Gulf of Gdańsk.

In 1999, the round goby was caught near Rugia (Corkum et al., in press). There are no further observations from that region but during the last two years several occurrences of round goby were noticed in the Pomeranian Bay (∼300 km west of the Gulf of Gdańsk). Data from the Gulf of Gdańsk provides information that makes it possible to address some important ecosystem features promoting successful invasion of the round goby. It is crucial to have no or little predation in the shallow water zone. Occurrence of solid bottom is necessary for effective breeding, but as in Gulf of Gdańsk, it is sufficient when solid elements occur in areas dominated by sandy bottoms. Bivalves are important food items for the round goby, but arthropods constitute an effective alternative food source. As the round goby does not migrate far, except during the cold season, transport in ballast waters is an effective means of establishing new invasions of round gobies. It is impossible to predict the time and place of the next round goby invasion in the Baltic Sea, but since the round goby has spread throughout the Gulf of Gdańsk in nine years, invasions of round goby elsewhere in the Baltic Sea in the future are assumed.

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Baltic Sea eutrophication: area-specific ecological consequences

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Key words: eutrophication, nutrient over-enrichment, ecological consequences, conceptual models, Baltic Sea

Abstract

Eutrophication of coastal waters is a global phenomenon. the amounts of nutrients in the brackish water of the Baltic Sea have increased several times during the last century, with severe ecological effects on the biota. With the increasing environmental problems caused by nutrient over-enrichment, public awareness to the problem has also risen. The Baltic Sea cannot be regarded as a uniform water mass, and area-specific ecological responses can be described. Changes in and detection of eutrophication-related parameters are discussed in relation to a generalized conceptual eutrophication model for the Baltic Sea. The cascading trophic and ecosystem-responses to eutrophication in 9 different sub-regions of the Baltic Sea are illustrated and discussed. The results clearly show the need not only for a common remedy for the Baltic Sea, but primarily show the importance of regional ecological assessment in relation to basin-wide eutrophication.

Introduction

Eutrophication has become a widespread matter of concern especially in coastal and inland waters during the last 50 years. Definitions, causes and consequences of eutrophication are explained and discussed in numerous papers, e.g. Gray (1992), Nixon (1995), Jørgensen & Richardson (1996) and Cloern (2001). The correlation between the eutrophication phenomenon and the brackish water of the Baltic Sea is also well studied and summarised in 'Marine eutrophication', a special issue of Ambio [19(3), 1990], as well as in Jansson & Velner (1995), HELCOM (1996, 2001), Rönnberg (2001) and http://www.grida.no/boing.

The Baltic Sea drainage area is densely populated, with over 85 million inhabitants in 14 countries, and the Helsinki Commission for protection of the marine environment of the Baltic Sea (HELCOM) has identified over 100 environmental hotspots within the catchment area (http://www.helcom.fi/pitf/ hotspots.html). The amount of nutrients and the deposition of organic matter in the Baltic Sea have increased considerably since the beginning of the 20th century (Larsson et al. 1985). According to Stålnacke (1996), 1 360 000 tons of nitrogen and 59 500 tons of phosphorus are annually discharged to the Baltic Sea through riverine load, coastal point sources, atmospheric deposition and nitrogen fixation.

A rising public and political awareness of the situation is seen in a variety of reports from governmental authorities in the countries around the Baltic Sea. Hence, Kauppila & Bäck (2001) for Finnish coastal waters, Anon. (2001) for the state of the Swedish coast, and Christensen (1998) for the Danish marine environment give detailed descriptions of the current state of these waters, primarily in relation to eutrophication. The programme BERNET (Baltic Eutrophication Regional NETwork) has evaluated and recommended measures for 7 restricted regions around the Baltic in BERNET (2000a, b). This awareness is also documented in Elmgren (1989), Partanen-Hertell et al. (1999) and in 'Man and the Baltic Sea' a special issue of Ambio [30(4–5), 2001]. The actual state of our knowledge of the processes affecting eutrophication in the Baltic Sea, how authorities and decision-makers can accurately improve the present situation, and cost-effective ways to achieve positive results are discussed in Bonsdorff et al. (2002), Elliott & de Jonge (2001) and Wulff et al. (2001).

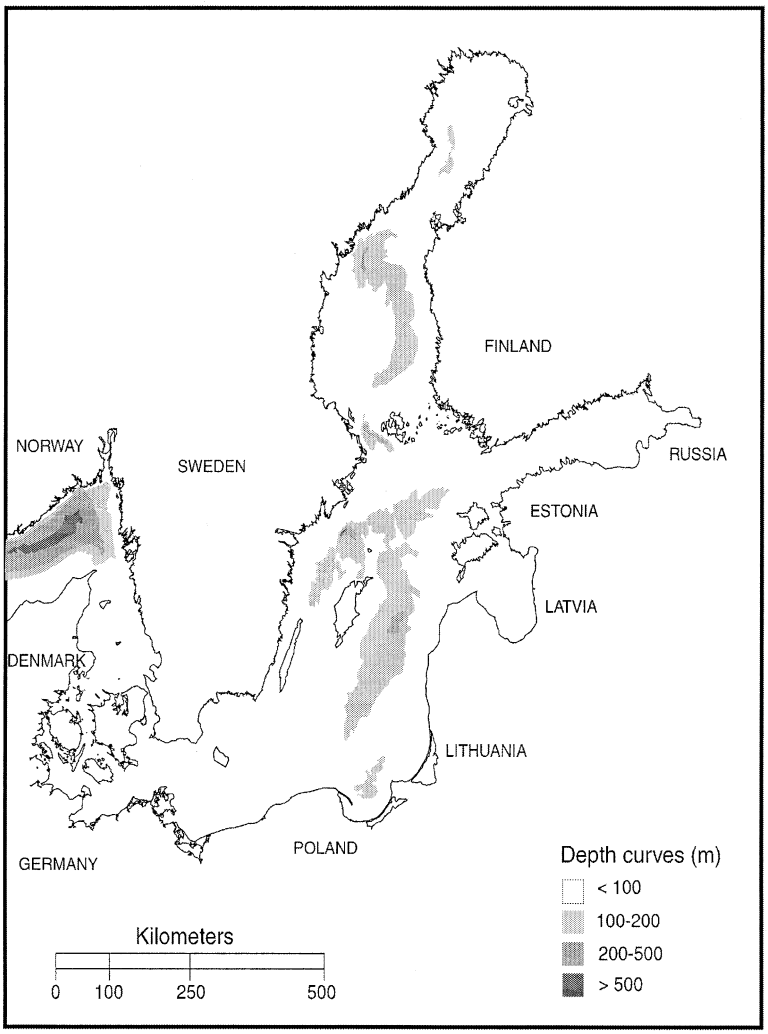


Figure 1. The Baltic Sea area.

For successful results in the rehabilitation of the Baltic Sea in the future, the background and consequences of eutrophication must be analysed and evaluated. This study describes the pathways of the eutrophication process in the Baltic Sea in a region-specific manner. The basic assumption was that all regions in the Baltic respond similarly to the same type of environmental stress (nutrient over-enrichment), but reality shows that local variations in the secondary (i.e. biological and ecological) responses of the ecosystem are important. Only if we describe and recognize them individually, can we present realistic measures to counteract the negative trends (Wulff et al., 2001; Bonsdorff et al., 2002).

The Baltic Sea – not an uniform water body

The Baltic Sea (Fig. 1) has an area of 415 000 km^2 with large variances and gradients in topography, geology, hydrography and climate (Leppäkoski & Bonsdorff 1989). The salinity gradient provides the basic difference between the southern and the northern part. There are also significant environmental variations between the coastal areas and the open sea, and between the archipelagos and the open coasts. All these large-scale patterns contribute to the fact that the Baltic Sea cannot be regarded as a uniform water body. The consequences of eutrophication take different pathways in different parts of the sea, with hypoxia and secondary effects on the benthic ecosystem as common problems, regardless of region (Karlson

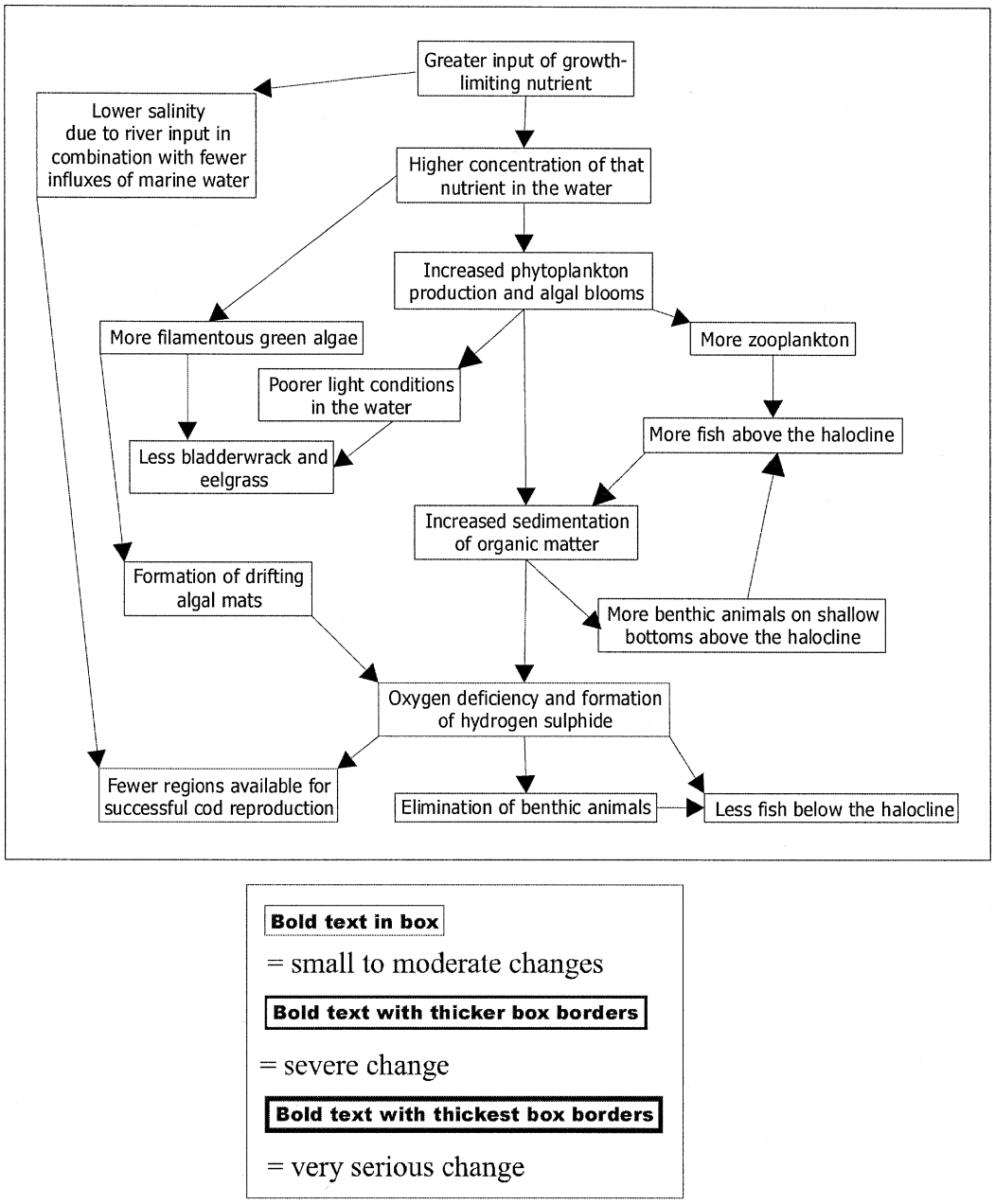
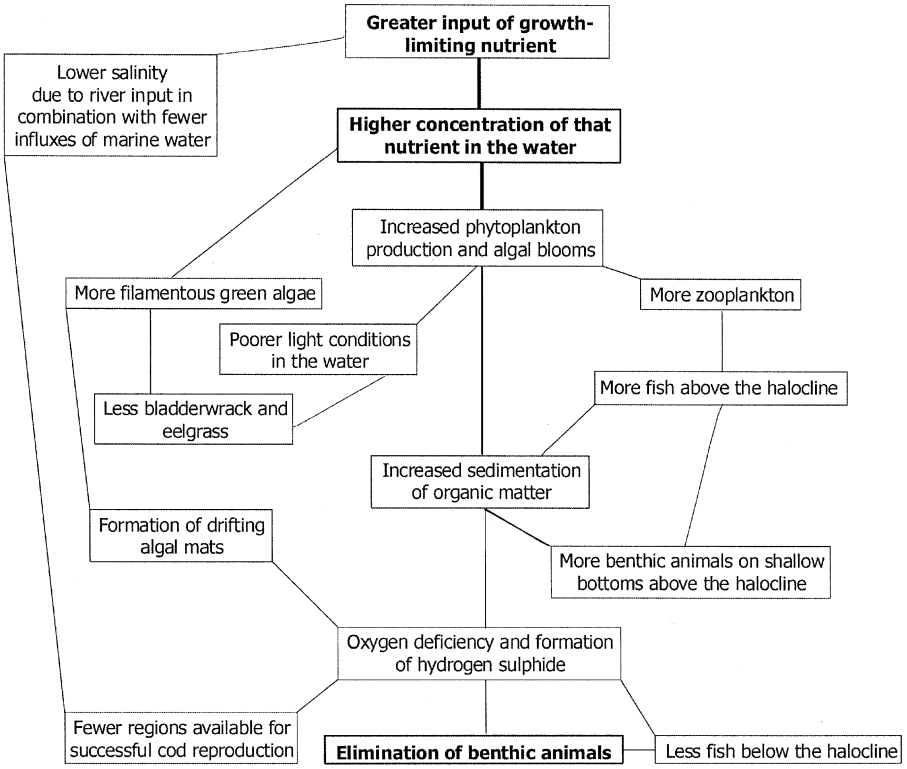

Greater input of growth-limiting nutrient
Lower salinity due to river input in combination with fewer influxes of marine water
Higher concentration of that nutrient in the water
Increased phytoplankton production and algal blooms
More filamentous green algae
More zooplankton
Poorer light conditions in the water
Less bladderwrack and eelgrass
More fish above the halocline
Increased sedimentation of organic matter
Formation of drifting algal mats
More benthic animals on shallow bottoms above the halocline
Oxygen deficiency and formation of hydrogen sulphide
Fewer regions available for successful cod reproduction
Elimination of benthic animals
Less fish below the halocline
Bold text in box
= small to moderate changes
Bold text with thicker box borders
= severe change
Bold text with thickest box borders
= very serious change

Figure 2. A conceptual flow-model of the eutrophication process (after Bernes 1988, Bonsdorff et al. 1997a, and Rönnberg 2001).

et al., 2002). For successful action plans and for real improvements to take place, we need information from each specific area of concern, and on the ecological effect-parameters that have undergone changes and for how long. For this analysis of potential regional differences in ecosystem responses to eutrophication (regardless of the sources of nutrient over-enrichment) in the Baltic Sea, we divided the area into 9 sub-regions, based on topographical, hydrographical and ecological considerations (Bonsdorff et al., 2002): The (1) Gulf of Bothnia, (2) Archipelago region, including the Archipelago Sea, Åland Islands and the Stockholm archipelago, (3) Gulf of Finland, (4) Gulf of Riga, (5) Gulf of Gdansk, including the Curonian and Vistula Lagoons, (6) East Coast of Sweden, from the Askö area in the north to Hanö Bay in the south, (7) Cent-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Oxygen deficiency and formation of hydrogen sulphide
Fewer regions available for successful cod reproduction
Elimination of benthic animals
Less fish below the halocline

Figure 3. Gulf of Bothnia. Potential effects of eutrophication.

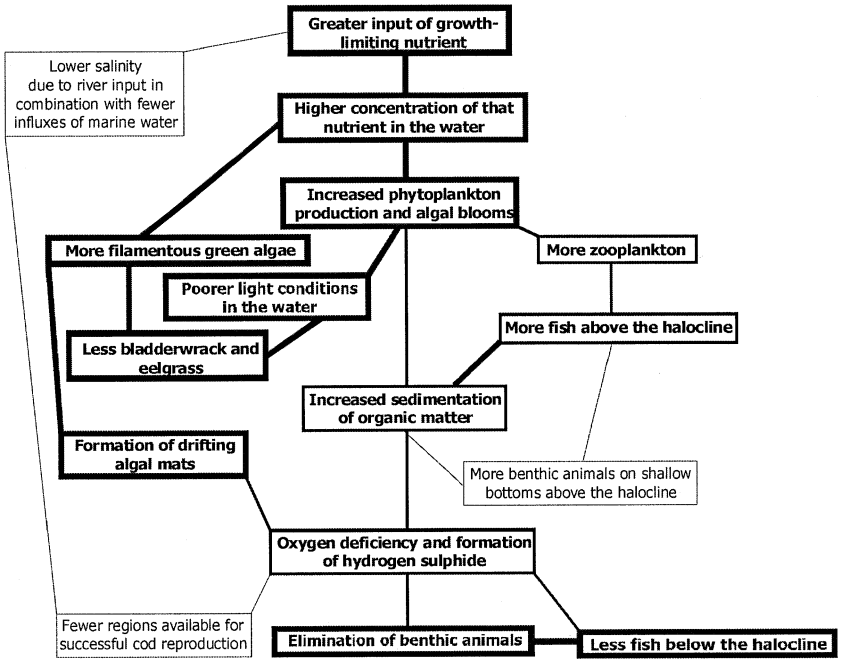
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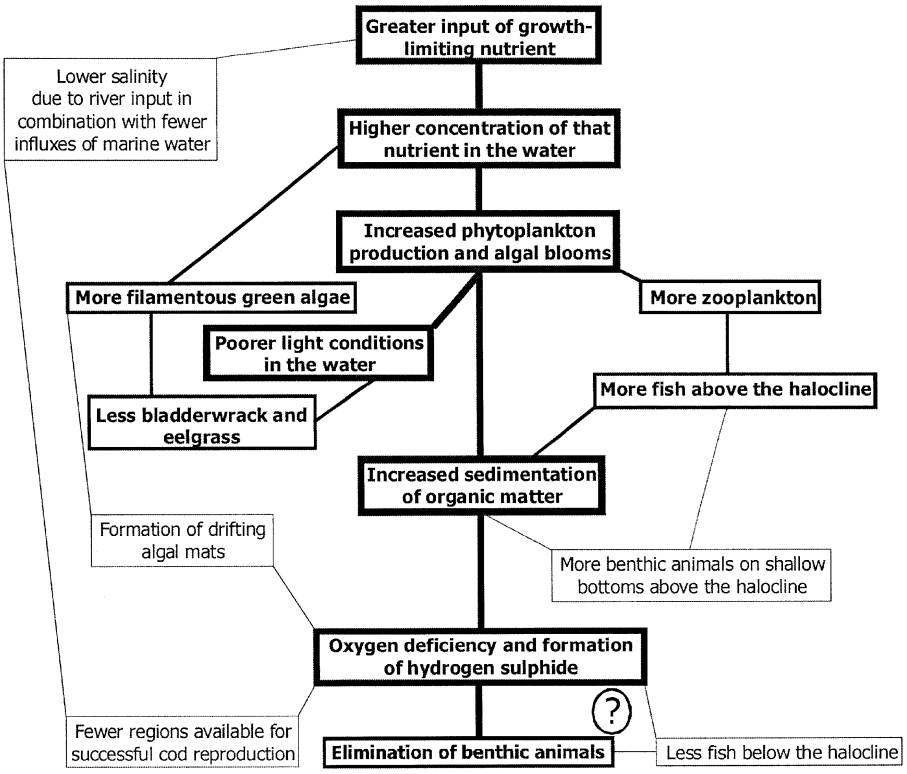
Figure 4. Archipelago region. Large-scale consequences of long-term eutrophication.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Oxygen deficiency and formation of hydrogen sulphide
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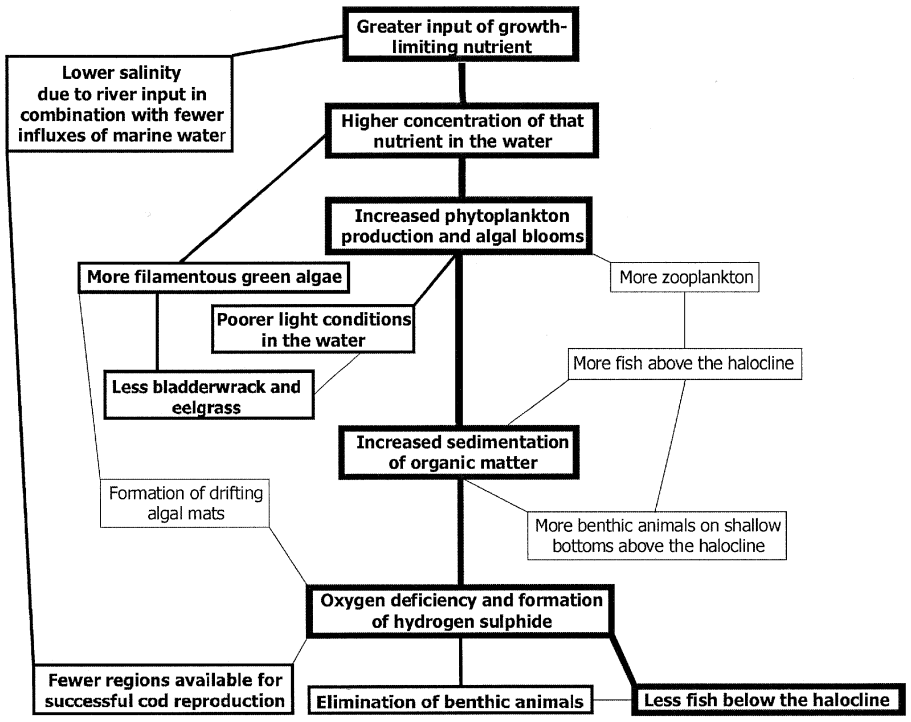
Elimination of benthic animals
Less fish below the halocline

Figure 5. Gulf of Finland. Large-scale consequences of long-term eutrophication.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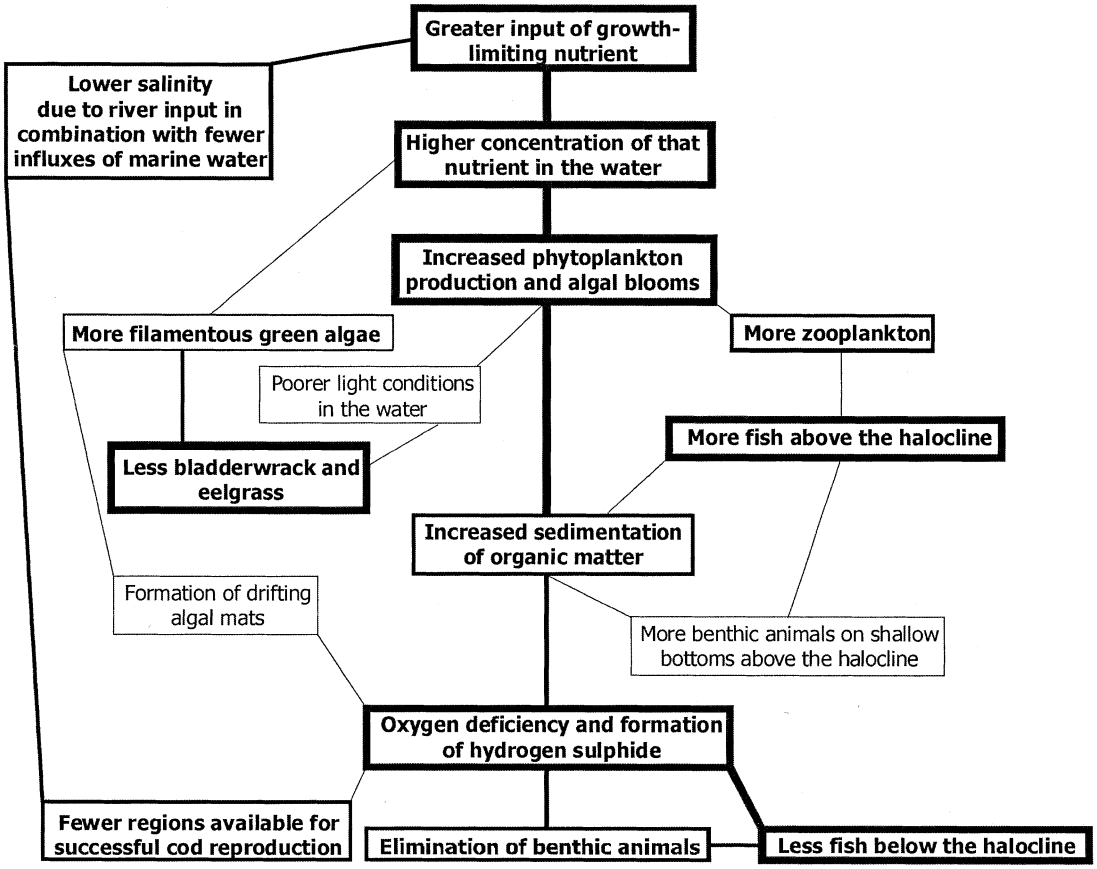
Oxygen deficiency and formation of hydrogen sulphide
Fewer regions available for successful cod reproduction
Elimination of benthic animals
Less fish below the halocline

Figure 6. Gulf of Riga. Consequences of regional eutrophication.

Table 1. Characteristics for the nine designated sub-areas of the Baltic Sea.

Area	Specific characteristics
Gulf of Bothnia	– consists of two sub-basins, Bothnian Sea (mean depth: 68 m, max. 230 m) and Bothnian Bay (mean depth: 43 m, max. 147 m) separated by the Northern Quark – salinity: 1–6 psu – land uplift: 8–9 mm yr^{-1} in the northern Bothnian Bay – days with ice-cover: 60 in the Bothnian Sea, 120 in the Bothnian Bay
Archipelago region	– includes Archipelago Sea, Åland Island, Åland Sea, and Stockholm archipelago ∼30.500 islands on the Finnish side, ∼30.000 islands on the Swedish side, separated by the Åland Sea – mean depth: 23 m, max. 294 m in the Åland Sea – salinity: 5–7 psu
Gulf of Finland	– direct extension of the Baltic proper, mean depth: 38 m, max. 120 m – salinity: 3–6 psu – days with ice-cover: 30 in the western gulf, 120 in the eastern – largest source of nutrients: runoff from River Neva
Gulf of Riga	– semi-enclosed, mean depth: 26 m, max. ∼60 m – salinity: 5 psu – largest source of nutrients: runoff from River Daugava
Gulf of Gdansk	– includes the open Gulf of Gdansk and the lagoons of Curonia and Vistula – mean depth: 3 m, max. 9 m – salinity: 5–7 psu in the Gulf of Gdansk, 0.5–6.5 in the lagoons – large catchment area with runoff from River Vistula
Swedish East-coast	– coastal area – includes studies especially from Himmerfjärden (mean depth: 17 m), St. Anna's archipelago and Hanö Bay – salinity: 5–11
Central Baltic	– consists of 5 deep basins – Arkona, Bornholm, Gdansk, Eastern- and Northern Gotland – separated by shallow sills – max. depth: 459 m (Landsort Deep in the Northern Gotland basin) – salinity: 6–16 psu (highly dependent of influxes of marine water from the North Sea)
Belt Sea region	– consists of Great- and Little Belt, Sound, Kiel Bight, Fehmarn Belt, Mecklenburger- and Pomeranian Bights – mean depth: 16–17 m, max. 80 m (in Little Belt) – salinity: 9–20 psu
Kattegat	– transition area between the Baltic Sea and the Skagerrak (North Sea) – mean depth: 23 m – salinity: 12–30 psu at surface, 32–34 psu at bottom – tidal amplitude: 20 cm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Oxygen deficiency and formation of hydrogen sulphide
Fewer regions available for successful cod reproduction
Elimination of benthic animals
Less fish below the halocline

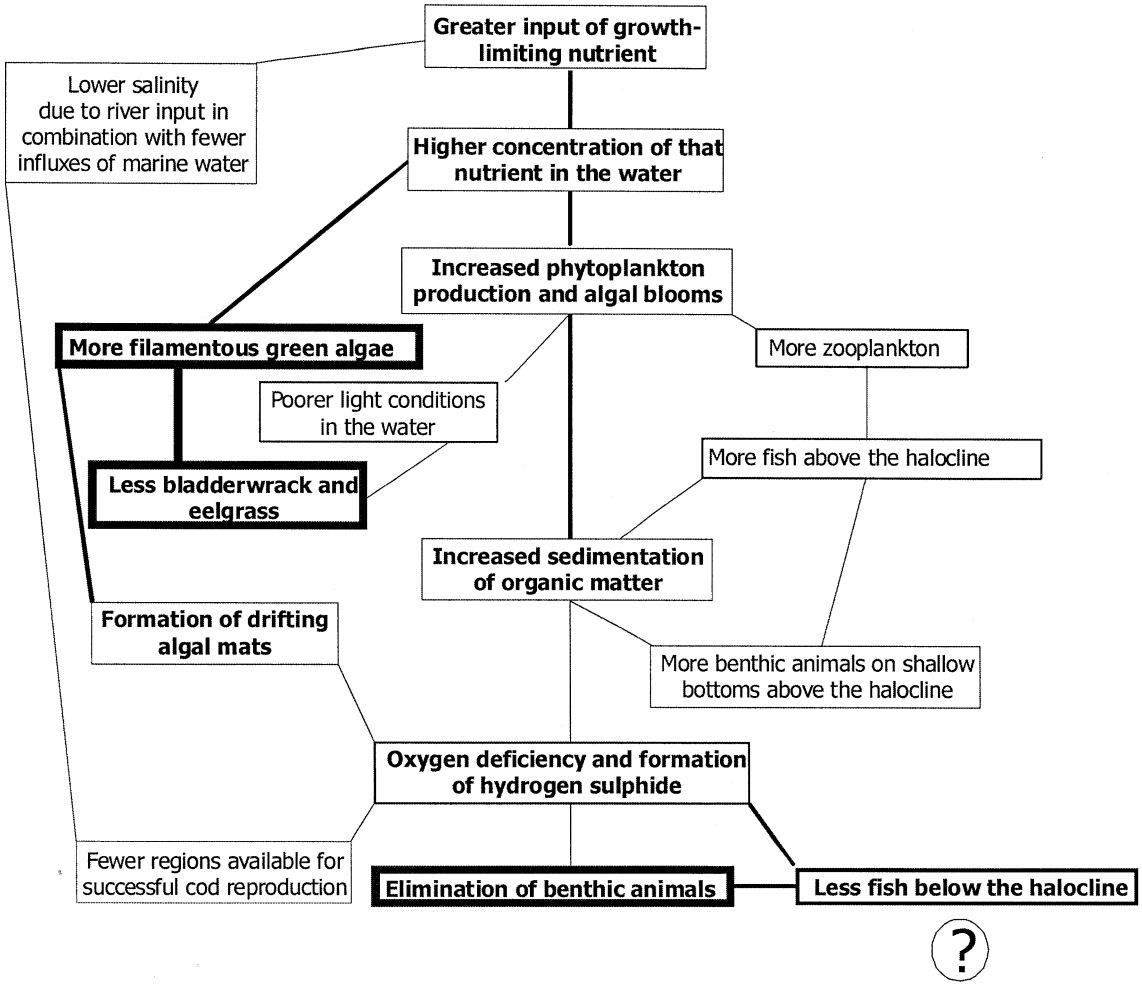
Figure 7. Gulf of Gdansk. Consequences of regional eutrophication.

ral Baltic, (8) Belt Sea region, including the Belt Sea and the Sound, Fehmarn Belt, and the bights of Kiel, Mecklenburg and Pomerania, and (9) Kattegat. Specific features for each region are presented in Table 1. For a detailed list of environmental and ecological features of the Baltic Sea, see, e.g. Leppäkoski & Bonsdorff (1989).

A more comprehensive description relating to eutrophication including characteristics of the areas, and changes for a variety of parameters (oxygen, nutrients, primary production and chlorophyll *a*, phytoplankton and algal blooms, macrovegetation, zoobenthos and fish) is found in Rönnberg (2001). An extensive review of the state of the zoobenthos in relation to eutrophication and hypoxia in the Baltic Sea area is presented in Karlson et al. (2002), and an analysis of the coupling between eutrophication and macroalgal assemblages is presented in Dahlgren & Kautsky (2001). The analysis of the parameters described for each region is based on public and available information, with special consideration to the access and availability to the sources.

Visualization of responses to eutrophication by conceptual models

A general conceptual model of the consequences of eutrophication is sometimes used to describe how an aquatic ecosystem is affected by an excess input of nutrients. Figure 2 shows an example of such a principal box model, modified after Bernes (1988) and Bonsdorff et al. (1997a), illustrating the main patterns of ecosystem responses above and below the semi-permanent halocline in the central Baltic Sea. By using this kind of generalized flow-charts for every sub-area, and by stressing the factors and processes that have undergone most changes in each area, one can obtain comprehensive summaries for any studied part of the Baltic Sea, provided there is published information available (Wulff et al., 2001; Bonsdorff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?

Figure 8. Swedish East-coast. Consequences of regional eutrophication.

et al., 2002). As the Baltic Sea is a heterogeneous water body, exact or uniform (chemical, biological or ecological) standards for water- and environmental quality are hard to define (cf. the demands of the EC Water Framework Directive (EC WFD 2000/60/EC, at http://europa.eu.int/comm/environment/water/index.html). Guidelines for assessing the extent of the changes are mainly based on the environmental quality parameters such as in Anon. (1999) suggested for the coastal waters of Sweden. The presented evaluations of the sub-regions of the Baltic Sea (Figs 3–11) are not quantitative, but rather qualitative assessments and summaries, based on available published and on-line information [see Bonsdorff et al. (2002) for further details on the sources]. Table 2 presents the basic quality-criteria followed in constructing the conceptual models, and serves as guidelines for interpreting the box-models. Transparency, oxygen concentration, nutrients, chlorophyll *a* and occurrences of harmful algal blooms (HAB) are easy to measure, and some long-term data is generally available. The problem is rather to generalize the overall assessment. The severity of the changes may differ between different regions. The values change seasonally and depend on what the pristine or unaffected conditions were like (defined as 'reference conditions' under the EC WFD). To evaluate the status of macrovegetation, zoobenthos and fish in relation to environmental change and altered biotic conditions is far more complicated, as these factors can be regarded as secondary effects, affected by the other components analysed. For example, factors as behavioural and physiological responses in the organisms to the changes in the environment may be specific for a given situation (Rabalais

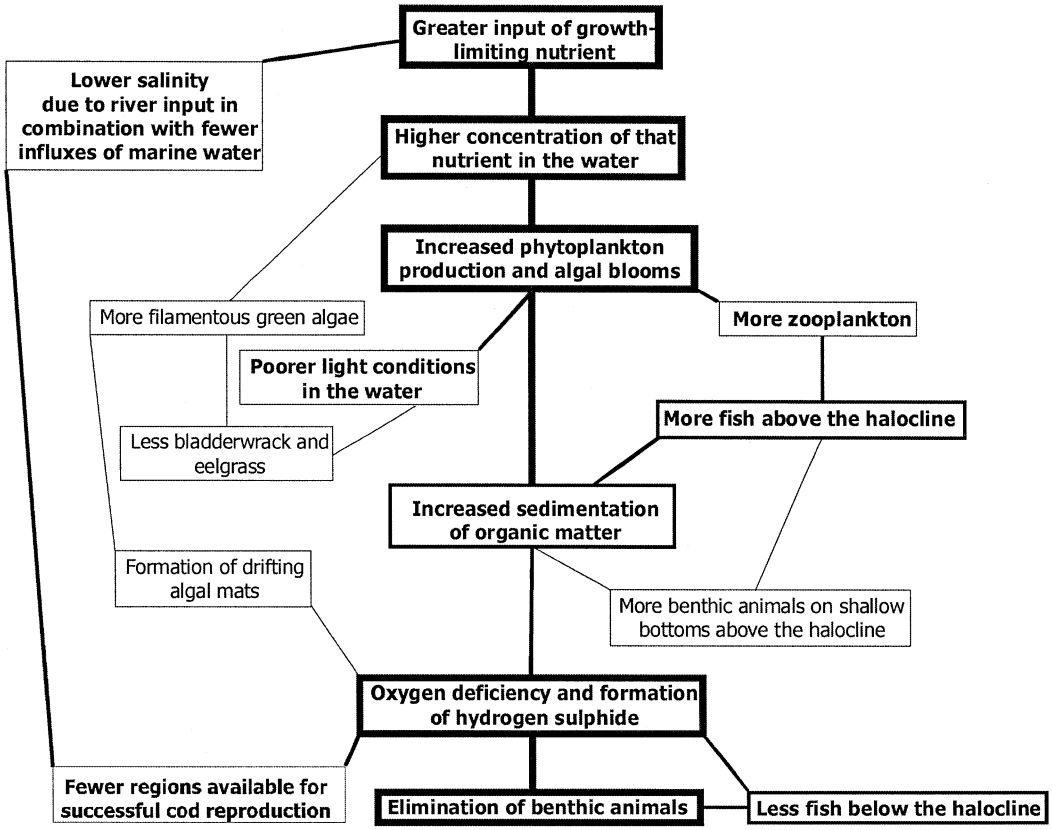
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Figure 9. Central Baltic. effects of eutrophication in the open Baltic Sea Waters.

& Turner, 2001). It is also hard to specify the portion of the community responses that are due to *eutrophication* in a multiple-stress situation, such as in the Baltic Sea (Gunnarson et al., 1995; Skei et al., 2000). A variety of other factors will also influence the biota, such as variations in oxygen, salinity and climate, pollutants and contaminants, traffic, introduction of non-native species, and overfishing. This holistic approach is presented in the status-reports by HELCOM (1990, 1996, 2001).

Area-specific patterns of ecosystem-responses to eutrophication

The identified pathways of eutrophication in the 9 subareas of the Baltic Sea are presented in Figs 3–11. From these graphs, the following general patterns, effects and consequences of long-term eutrophication can be identified for each individual region:

(1) *Gulf of Bothnia*

During the last 30 years, a slight increase in the concentration of nutrients in the Gulf of Bothnia has taken place (HELCOM, 1996; Karjalainen, 1999). Overall, however, there are no clear signs of eutrophication (Fig. 3). Contributing reasons are the open character of the Gulf with an effective water exchange (Kirkkala, 1998). Some industries around the coast act as local point sources (HELCOM, 1996). There is a potential risk that problems related to eutrophication will appear as increased frequencies and magnitudes of pelagic offshore harmful algal blooms, and as increased problems with loose lying filamentous algal mats in near shore and coastal waters.

(2) *Archipelago region*

The Archipelago region is one of the most studied areas in the Baltic Sea, and therefore the processes of eutrophication are well documented (Bonsdorff et al., 2002). These complex and shallow (mean depth only 23 m) areas act as potential 'filters' for nutrients

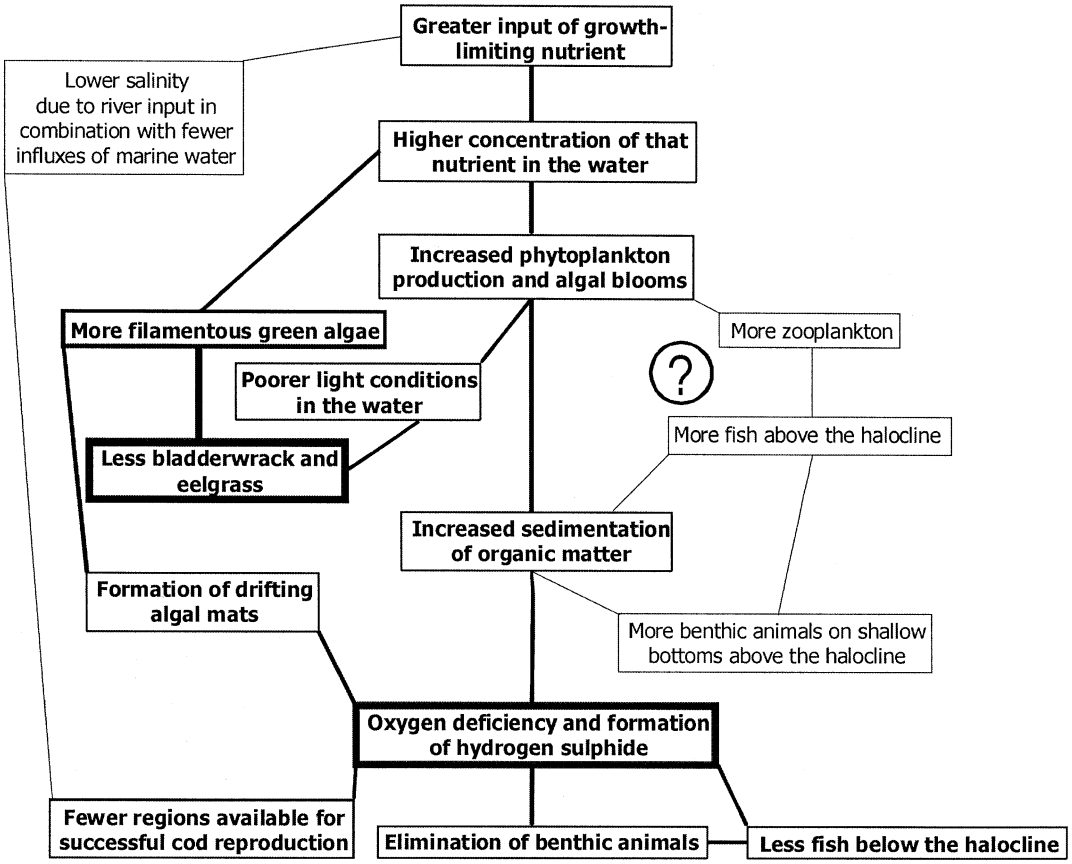
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Figure 10. Belt Sea region. Eutrophication-related effects on coastal ecosystems.

and particles between the coastline and the open sea, and also between the Baltic proper and the Bothnian Sea (Jumppanen & Mattila, 1994; Bonsdorff et al.,1997a,b). As is shown in Fig. 4, the consequences of diminishing (locally disappearing) macrovegetation (perennial brown algal belts; *Fucus vesiculosus*) and the formation of dense benthic filamentous algal mats (Vahteri et al., 2000) have given rise to large changes. Changes in community composition and in zoobenthic and fish populations are also detected (Bonsdorff et al., 1997a,b; Kauppila & Bäck, 2001). In this area it is evident that nutrient overenrichment has given rise to severe and cascading trophic effects throughout the ecosystem, leading to acute demands for management- and restoration plans.

(3) *Gulf of Finland*

The Gulf of Finland is regarded as one of the most polluted areas of the Baltic Sea (HELCOM, 1990). The Russian multimillion city of St. Petersburg and the River Neva estuary are the main, but not the only, sources of nutrient input. The lack of thresholds at the entrance of the Gulf of Finland makes the area a direct extension of the Baltic proper. As the water has an anticlockwise circulation, this results in an eastward transport along the Estonian coast, and a westward transport along the Finnish coast (HELCOM, 1996). This means that the Finnish coast is most exposed to nutrients transported from local sources around the entire Gulf. Harmful algal blooms and problems due to hypoxia are the topics of special concern in the Gulf of Finland (Fig. 5). Recently, modelling efforts have been performed to assist in planning remedies for this region (Kiirikki et al., 2001), but for the coastal waters, the total extent of the problem has only lately been described (Kauppila & Bäck, 2001). The problems are further complicated by the fact that in the inner Gulf, the system is phosphorus-limited, whereas the entrance of the Gulf shows clear nitrogen-limitation (Pitkänen, 1991). This is reflected in correlations between pelagic primary production

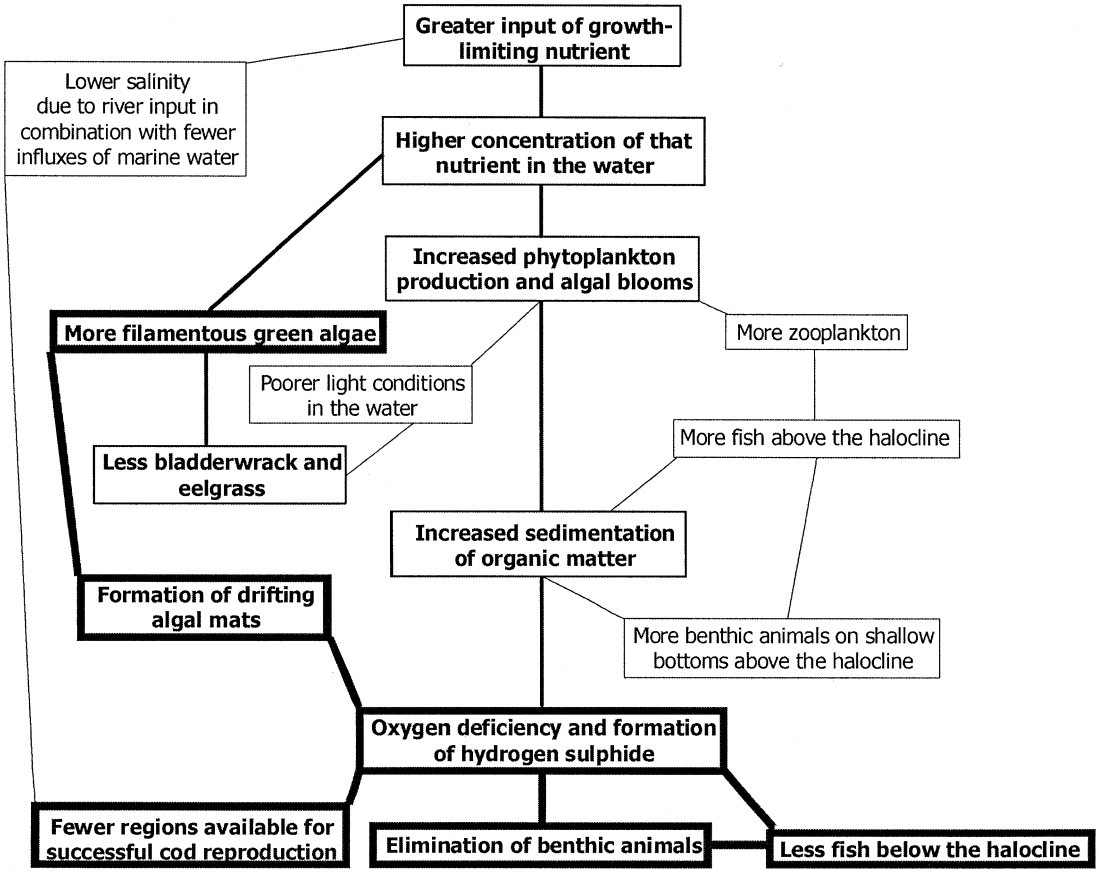
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Figure 11. Kattegat. Marine eutrophication in the mixing zone between the Baltic Sea and the North Sea.

(chl-*a*) and phosphorus, nitrogen, oxygen concentrations in the bottom water, and Secchi depth of the water body. With increasing P-limitation (inner Gulf), primary production shows increasing positive relationships to phosphorus and oxygen, whereas towards the open Baltic Sea, there is a positive correlation to nitrogen, but an increasing negative correlation to transparency (Rönnberg, 2001).

(4) *Gulf of Riga*

The Gulf of Riga has a small volume and restricted water exchange, which makes it sensitive to pollution and other local sources of stress (Fig. 6). On the other hand, the Gulf has a large buffering capacity. The flat topography and extensive wetland areas on land together with a long retention time in the soil and groundwater prevent the nutrients from direct entry to the Gulf. In a Nordic Environment Research Programme 1993–1997, the Gulf of Riga was thoroughly studied, and the results showed that the nutrient concentrations in the area have been relatively stable during the 1990s (Wassmann & Tamminen, 1999). The most concrete sign of eutrophication is seen on the fish populations. The Gulf of Riga has been one of the most important fishing areas in the Baltic (Ojaveer, 1995). There has been an increase in the mortality of herring (*Clupea harengus membras*) embryos, disturbed reproduction and nursery areas for eelpout (*Zoarches viviparus*) and smelt (*Osmerus eperlanus*), while the populations of perch (*Perca fluviatilis*) have increased (Ojaveer & Gaumiga, 1995). Also, increased occurrences of annual filamentous algae affect the ecosystem (Kotta & Orav, 2001).

(5) *Gulf of Gdansk*

The Gulf of Gdansk, and especially the Puck Lagoon, has been rich and diverse in macrophytes. In the 1950s 80 taxa were present, dominated by *Fucus vesiculosus* and *Furcellaria lumbricalis*. Eutrophication-related responses, such as increase in nutrient-levels

Table 2. Guidelines for the degree of changes in all the parameters considered in relation to the scale of changes in the conceptual flow-models. the estimates are based on standards in Jumppanen & Mattila (1994), Rumohr et al. (1996), Anon. (1999), and Dahlgren & Kautsky (2001). Note that the values for the six first parameters represent summer values (August value for chlorophyll *a*).

Parameter	Effects: Small-moderate	Severe	Very serious
Transparency	3–5 m	2–3 m	<2 m
Oxygen	4–6 ml l^{-1}	2–4 ml l^{-1}	<2 ml l^{-1}
Tot-P	15–19 μg l^{-1}	19–24 μg l^{-1}	>24 μg l^{-1}
Tot-N	250–310 μg l^{-1}	310–360 μg l^{-1}	>360 μg l^{-1}
Chlorophyll *a*	1.5–2.2 μg l^{-1}	2.2–3.2 μg l^{-1}	>3.2 μg l^{-1}
Harmful Algal Blooms (HAB)	Few colonies	Formation of floating algae	Bloom-areas and layer of cyanobacteria
	1–5 g m^{-3}	5–11 g m^{-3}	>11 g m^{-3}
Macrovegetation	*Fucus* species, meadows of *Zostera* with associated charophytes, relatively sparse abundances of filamentous algae.	Filamentous algae as epiphytes on *Fucus*, sporadic occurrences of *Zostera*, no charophytes. Filamentous algae dominate.	No *Fucus* present. Filamentous algae dominate. Drifting algal mats and sulphur-bacteria.
Zoobenthos	Dominated by molluscs and long-lived polychaetes. Increased total biomass/production. Low species richness, high adundance, low biomass.	Animals live close to or at the sediment. Small worms (e.g. *Capitella capitata*) dominate.	No macrofauna. Lack of bioturbation, lamination of the sediment.
Fish	Decrease in flatfishes.	Decrease in cod, increase in herring, sprat and cyprinides.	

through discharges from land, and consequently decreased transparency, have dramatically altered the environment. In the beginning of the 1990s, only 28 macrophyte taxa were left, and fast growing filamentous and annual species of *Ectocarpus* and *Pilayella* dominated on 70% of the bottoms (Fig. 7). The diminishing underwater meadows of *Zostera marina* and of perennial (brown) algae have further affected the fish communities in the Gulf. The commercial species, such as eel (*Anguilla anguilla*) and pike (*Esox lucius*) have decreased, and been replaced by cyprinids, such as roach (*Rutilus rutilus*) (Ciszewski et al., 1991, 1992). Local remedies have been implemented, and a slow recovery of the ecosystem seems to be taking place (Rönnberg, 2001).

(6) *East coast of Sweden*

The East Coast of Sweden is a long coastal area where mainly local point sources contribute to eutrophication. Most changes are seen in the biomass of macrovegetation (Fig. 8). Increases in the amounts of filamentous algae coupled to decreased transparency have lead to reductions and even disappearances of *F. vesiculosus*. The reductions in bladder wrack further affect the fauna associated with the vegetation, through food deficiencies for grazers and loss of nursery areas (Wulff & Hallin, 1994; Kautsky, 1998), and there seems to be a coupling between eutrophication, pollution and local failure of fish recruitment (Andersson et al., 2000).

(7) *Central Baltic*

The Central Baltic consists of some deep basins separated by shallow sills, restricting circulation and causing periodic stagnation (HELCOM, 1996; Karlson et al., 2002). The occurrences of cyanobacterial blooms in the Central Baltic have increased since the 1960s, mainly due to the increase in anthropogenic nutrients in the water. Warm and calm conditions in July and August further aggregate the cyanobacteria in the surface water (Finni et al., 2001 a). Cyanobacterial blooms are, however, no new phenomena in the Baltic Sea. According to Bianchi et al. (2000) blooms have occurred since the present brackish water phase of the Baltic was developed 7000 years B.P. The accumulation of organic material on the bottoms enhances the oxygen consumption, leading to hypoxia in the bottom waters, causing mortality among benthic animals (Fig. 9). The hypoxia or anoxia is, however, also a consequence of rare influxes of oxygenated, saline and subsequent prolonged stagnation periods of the water (HELCOM, 1996).

(8) *Belt Sea Region*

The effects of eutrophication in the Belt Sea Region are mainly seen as a reduction in geographic and in depth distribution of macrovegetation, in oxygen deficiency, and in formation of hydrogen sulphide (Fig. 10). In the Danish waters substantial reductions in the abundances of *Zostera marina* are observed as is reported from the Kiel Bight and the Greifswalder Bodden in the Pomeranian Bight, where also brown- and red-algal species have been reduced (e.g., Anon., 1991; Messner & von Oertzen, 1991; HELCOM, 1996). From Danish waters positive signs to measures taken can be seen, stressing the importance of broad ecological advisory programmes (Conley et al., 2002).

(9) *Kattegat*

The strong halocline in the Kattegat (Fig. 11) increases the rate of primary production in the surface waters, and the stratification of the bottom waters, which leads to regional hypoxia (Karlson et al., 2002). The hypoxia affects the zoobenthos and the ichthyofauna negatively: the Norway lobster, *Nephrops norvegicus*, and young flatfish are examples of species affected (Baden et al., 1990). The increased amounts of filamentous algae, primarily green algae, cause drifting mats before decomposition (Nielsen & Dahl, 1992; Pihl et al., 1999). This also contributes to the poor bottom conditions, and to habitat deterioration.

General patterns and measures

Irrespective of the area-specific effects of the increased loads of nutrients to the Baltic Sea, the sources are more or less similar in the whole region. The extent and the severity of the discharges may differ, however. As is seen in e.g. HELCOM (1996) and Rönnberg (2001), the major sources in the input of nutrients are derived from agriculture, industry, municipal sewage and transports. Nitrogen emissions in form of atmospheric depositions are also important, as well as local point sources, such as aquaculture and leakage from forestry.

A reduction in nutrients from agricultural runoff and industrial production has been seen in all the former communist states in Eastern Europe. This is mainly due to the collapse in economy after the end of the Soviet Union era. The use of mineral fertilizers has also decreased in the market economic countries during the 1990s as a consequence of national legislation for a more environmental-friendly agriculture (Karjalainen, 1999). It may, however, take time before any positive ecological effects are seen. Stores of nutrients in soil, groundwater and sediments must be depleted before real reductions in the water environment are detected (Conley et al., 2002). Loading from industries and other point sources is less complicated to manage than the municipal sewage water or agricultural runoff, which can be more diffuse. Scattered dwellings on the countryside are impossible to connect to wastewater treatment plants, and the situation is therefore under better control in more densely populated areas. In the former socialist countries, the wastewater treatment is still under construction (Karjalainen, 1999), but progress is being made (Finni et al., 2001 b). In order to focus our efforts to combat eutrophication, it is important that we know the components and their linkage in any regional ecosystem that suffers from nutrient over-enrichment. Improvements in nutrient reductions in for example Sweden are presented in Elmgren & Larsson (2001).

Eutrophication is a serious problem in the entire Baltic Sea area. Despite the fact that more or less the same ecological parameters are involved in the processes in the different parts of the sea (Wulff et al., 2001; Bonsdorff et al., 2002), the effects and consequences may vary, which underlines the importance

of area-specific assessment and advice, measures and ecosystem rehabilitation. We must, however, be aware of the varying and potentially long time span before positive signs of reductions are seen in the aquatic environment.

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How does eutrophication affect different life stages of *Fucus vesiculosus* in the Baltic Sea? – a conceptual model

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Abstract

During the last few decades the perennial seaweed *Fucus vesiculosus* L. has rapidly declined in large parts of the Baltic Sea. Indirect effects of eutrophication, such as increased turbidity, sedimentation, grazing and occurrence of filamentous algae, have generally been suggested as major factors causing the decline. It is only recently, however, that the effects of these factors have been experimentally tested and here we summarise these results in a new conceptual model. In many areas, it might be desirable to enhance the recovery of *Fucus* artificially, as the natural rate of re-establishment of this important macroalga is limited both in time and space. To be able to optimise and evaluate the potential of restoring *Fucus* belts in eutrophicated areas, we need to know how eutrophication affects critical steps in its life cycle, such as the attachment, germination and early growth of germlings. We suggest that more attention should be given to the survival of early post-settlement stages in *Fucus* as they are crucial for the maintenance and re-establishment of populations. We also stress that the two reproductive periods that the species exhibits are included in discussions concerning effects of eutrophication on *Fucus*, as it is likely that the outcome of reproduction will differ between the two reproductive periods. In the new conceptual model we distinguish between the effects on adult and juvenile life stages and we add the two reproductive strategies of *Fucus*.

Background

During the last few decades numerous reports have described geographical and vertical decreases of *Fucus vesiculosus* in the Baltic Sea. Observations of diminishing or disappearing *Fucus* belts have been made in Finnish (Kangas et al., 1982; Haahtela, 1984; Kangas & Hällfors, 1985), Polish (Plinski & Florczyk, 1984; K. Skora, pers. commun.), German (Vogt & Schramm, 1991) and Swedish coastal areas (Kautsky et al., 1986; Eriksson et al., 1998; Engkvist et al., 2000). Conceptual models of the decline have been presented, suggesting that not only one, but rather a combination of factors have contributed to the decline (Kangas et al., 1982; Haahtela, 1984; Vogt & Schramm, 1991). Factors having negative impacts on *Fucus* populations are, for example, pollution (Rosemarin et al., 1986; Kautsky, 1992; Andersson & Kautsky, 1996; Wikström et al., 2000), grazing (Malm et al., 1999; Engkvist et al., 2000) and ice scouring (Waern, 1952; Haahtela, 1984; Kiirikki & Ruuskanen, 1996).

The most important factor affecting *Fucus* in the Baltic Sea has been ascribed to the indirect effects of eutrophication, such as increased turbidity and decreased light levels (Kautsky et al., 1986; Vogt & Schramm, 1991; Eriksson et al., 1998), increased sedimentation (Kiirikki, 1996; Schramm, 1996) and increased competition with filamentous algae (Kangas et al., 1982; Worm et al., 1999, 2000). It has long been suggested that eutrophication lowers the fitness of adult individuals (Kautsky et al., 1986) and prevents the establishment of juvenile *Fucus* (Schramm, 1996). However, few experimental studies have been conducted to evaluate these factors, and it is only recently that the effects of eutrophication on the early

life stages of *Fucus* have attracted attention (Berger, 2001; Bergström et al., 2003; Eriksson, 2002; Worm et al., 2000, 2001). The direct effect of increased nutrients on *Fucus* reproduction and early survival has so far not been included in discussions concerning the decline and maintenance of this important macroalga. The aim of this contribution is to summarise recent studies in the Baltic Sea concerning negative effects of eutrophication on different life stages of *F. vesiculosus*. Furthermore, we would like to suggest a new conceptual model to the factors affecting the populations of the Baltic *F. vesiculosus*, based on the present state of knowledge.

Recovery of *Fucus* populations

Because of the significant role of *Fucus vesiculosus* in the Baltic coastal ecosystem (Kautsky et al., 1992), a re-introduction of the species into areas where it has disappeared would be desirable. Although a natural recovery of *Fucus* populations has been observed locally along the Swedish east coast (Kautsky, 2000; Engkvist et al., 2002) and at the Finnish coast lately (S. Bäck, pers. commun.), an improvement of populations by artificial re-establishment may be necessary in other areas. The natural re-establishment of *Fucus* in the Baltic Sea may be very slow, because the reproduction is limited both in time (Bäck et al., 1991; Andersson et al., 1994; Berger et al., 2001) and in space (Serrão et al., 1997). Gamete release occurs in a circadian and lunar rhythm, with a peak release at 18:00 to 22:00 h 2 days before full and new moon (Andersson et al., 1994). In the Baltic Sea, *F. vesiculosus* releases its gametes during a short period during summer (May–June) (Bäck et al., 1991) or during a short period during autumn (September–November) (Berger et al., 2001). Gamete viability is restricted to a few hours after release (Serrão et al., 1999) and gamete dispersal is restricted to 0.5 m (eggs) and 2 m (sperm) (Serrão et al., 1997). Artificial re-establishments can take place either by transplanting adult reproductive plants attached to stones, or by seeding zygotes by transferring fertile structures (Nilsson et al., 1999; Berger, 2001). To be able to optimise management actions, we need to know how eutrophication affects the different life stages of *F. vesiculosus*.

Effects of eutrophication on different life stages

A summary of experimental studies and field surveys on the effects of eutrophication on *Fucus vesiculosus*, published since 1982 and onwards, is shown in Table 1. The viability and fitness of adult individuals of *Fucus* in the Baltic Sea is, in particular, negatively affected by decreased water transparency, by epiphytic load and by grazing by *Idotea baltica*. Recently, Hemmi & Jormalainen (2002) showed that the quality of *Fucus* as food for *Idotea* is improved by nutrient enrichment, suggesting that eutrophication enhances the grazing performance of *Idotea*. Although there is some indications that the numbers of *Idotea* increase upon nutrient enrichment (Worm et al., 2000), further studies are needed to verify whether the population sizes of *Idotea* and thereby the magnitude of grazing damage upon *Fucus*, may be increased by eutrophication. In contrast to this hypothesis, Worm et al. (1999, 2001) observed a positive effect of grazing on the recruitment of *Fucus* during nutrient enrichment. They showed that grazers favoured *Fucus* recruitment by selective consumption of annual algae. Thus, the effect of grazers on *Fucus* in eutrophic environments is not only negative, but may depend on the local characteristics, the life stage of *Fucus* and the magnitude of grazing.

Factors acting on the adult individuals may result in a decline and disappearance of established populations. The population maintenance and re-establishment of *Fucus* is, however, dependent on a few critical steps in the beginning of its life cycle, including fertilization, attachment and early post-settlement mortality (Vadas et al., 1992). During the last few years, we have conducted experimental studies aimed at testing the effect of some specific factors related to eutrophication on the early life stages of *F. vesiculosus*. These include the interspecific interactions between germlings of *Fucus* and filamentous annual algae (*Cladophora glomerata*) (Berger et al., 2003), the effects of deposited matter on attachment and early survival (Berger et al., 2003) and the direct effect of nutrient enrichment on the attachment, germination and rhizoid development process (Bergström et al., 2003). Negative effects on the settlement, either by space pre-emption or by delaying the attachment process, as well as on the germination was observed in all studies. To understand the actual structuring importance of these factors, large-scale studies *in situ* are necessary. Worm et al. (2001) have recently published such a study. In a factorial field experiment, they ma-

Table 1. A summary of factors and effects, related to eutrophication, known to influence *Fucus vesiculosus* in the Baltic Sea. Classification of studies as either experimental studies (E) or field surveys (S).

Factor	Effect	E/S	References
Decreased water transparency	Decreased depth penetration of *Fucus*	S	Eriksson et al., 1998 Kangas et al., 1982 Kautsky et al., 1986 Vogt & Schramm, 1991
Nutrient enrichment	Delayed attachment of fertilised eggs and reduced germination success at moderate and high concentrations of nitrate and phosphate	E	Bergström et al., 2003
	Single pulses (5 h) of nutrients favour fast-growing epiphytes, can cause decline of *Fucus*	E	Worm & Sommer, 2000
	Nutrient enrichment decreased *Fucus* cover and recruitment success, by favouring filamentous algae	E	Worm et al., 2000
Sediment	Attachment and early survival of germlings inhibited by deposited matter	E	Berger et al; 2003
	Fucus established only on substrate free of sediment	E	Eriksson, 2002
Filamentous annual algae	Attachment and early survival of germlings inhibited by filamentous algae	E	Berger et al., 2003
	Enteromorpha spp. blocked settlement and recruitment of *Fucus*	E	Worm et al., 2001
	Coverage of *Fucus* negatively correlated with cover of filamentous algae	S	Worm et al., 1999
	Epiphytes reduce viability and growth of adult *Fucus*	S	Kangas & Hällfors, 1985 Vogt & Schramm, 1991
		E	Worm & Sommer, 2000
Grazers	Increased populations of *Idotea baltica*, mediated by increased abundance of filamentous algae, may overgraze *Fucus* populations	S	Haahtela, 1984 Kangas & Hällfors, 1985
	Nutrient enrichment may improve the quality of *Fucus* as food, thereby cause overgrazing by *Idotea baltica*	E	Hemmi & Jormalainen, 2002

nipulated the presence of grazers and the propagule bank as well as nutrient supply. They found a strong negative interaction between epilithic annual algae and *Fucus* recruitment. *Enteromorpha* spp. acted as a settlement barrier that strongly interfered with *Fucus* recruitment. Furthermore, in a long-term sediment removal field experiment (Eriksson, 2002) showed that sedimentation has a major impact on the depth distribution of perennial macroalgae in the northern Baltic Sea. In their study, germlings of *Fucus* were only able to establish in areas cleared of sediment. The relative effect of the above-mentioned factors on *Fucus* recruitment is likely to depend on *local* differences in, for example, biomass and species of filamentous algae, sedimentation patterns and nutrient regimes. Additional factors, such as low salinity, may further increase *Fucus* susceptibility to eutrophication.

Impact by eutrophication on *Fucus vesiculosus* – dependent on season of reproduction?

In the Baltic Sea, *Fucus vesiculosus* exhibits two periods of reproduction (Berger et al., 2001). Summer-reproducing individuals of *Fucus* release their gametes during May and June (Bäck et al., 1991), whereas autumn-reproducing individuals release their gametes from September to November (Berger et al., 2001).

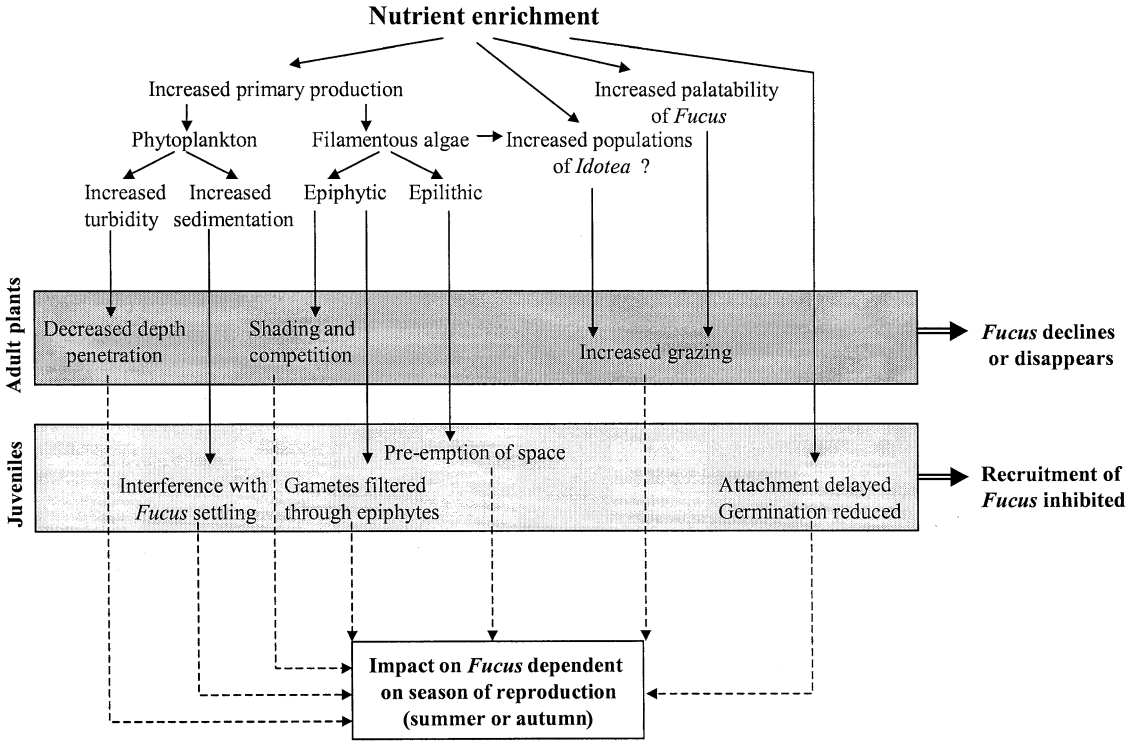

Figure 1. Conceptual model to factors affecting the decline and maintenance of *Fucus vesiculosus* in the Baltic Sea, including the two reproductive strategies. Effects on adult plants and on juveniles are shown in the dark and light grey boxes, respectively. (For references, see text and Table 1.)

Summer- and autumn-reproducing plants occur either separately or in mixed stands along the east coast of Sweden. It is plausible that the impact by eutrophication on *Fucus* recruitment will depend not only on local but also on *seasonal* differences in the occurrence of filamentous algae, sediment and nutrients and on *Fucus* reproductive strategy in a specific area. For example, filamentous algae have a higher abundance during spring and summer than during autumn (Kiirikki & Lehvo, 1997; Lotze & Worm, 2000; own observations). Sedimentation rates in coastal areas also exhibit large seasonal variation in quantity and quality (Wassmann, 1984; Heiskanen & Tallberg, 1999), with an annual maximum sedimentation rate often occurring during late spring in the Baltic Sea (Heiskanen & Leppänen, 1995; Heiskanen & Tallberg, 1999). These two facts suggest that there might be different preconditions for gametes settled during summer as compared with those settled during autumn. As hypothesised by Berger et al. (2001) and Worm et al. (2001) autumn-reproducing individuals of *Fucus* may have a selective advantage over summer-reproducing individuals because they may escape competition from summer annuals such as *Enteromorpha* spp., *Pilayella littoralis* and *Cladophora glomerata* and they may miss the peak of sedimentation. This advantage may result in an increased occurrence of autumn-reproducing populations in the Baltic Sea. To determine if an increased occurrence of autumn-reproducing populations will counteract the general decline of *F. vesiculosus* large-scale surveys are needed. The investigation of the geographical distribution of summer- and autumn-reproducing *Fucus*, which was conducted along the east coast of Sweden in 1996 (Berger et al., 2001), may serve as a starting point for further surveys.

Description of the conceptual model

Recent experimental studies suggest that, besides pollution, grazing and ice scouring (all unrelated to eutrophication), increased nutrients have negative impacts on the populations of *Fucus vesiculosus* in the Baltic Sea. Both adult and juvenile life stages of *Fucus* are negatively affected by nutrient enrichment,

as indicated by the dark and light grey boxes in the conceptual model (see Fig. 1). The recruitment and juvenile life stages of *Fucus* are likely to be the most sensitive to nutrient enrichment, being affected either indirectly (through interference with filamentous algae and sediment) or directly (through nutrient stress) (Bergström et al., 2003). The effects of eutrophication may act both in the long term, by hampering photosynthesis and thereby growth of adult plants caused by decreased water transparency, and in the short term (on time-scales of hours and days) by affecting the attachment and germination of *Fucus*, i.e. on critical steps in the *Fucus* life cycle. Rapid declines of *Fucus* populations may initially be mediated by factors acting on the adult plants, whereas recruitment and recovery of populations may be inhibited by factors acting on the attachment process and on the juveniles, thereby reducing *Fucus* capacity of natural re-establishments. The two reproductive strategies are also included in our conceptual model, as it is likely that the impact of eutrophication, as discussed above, will differ depending on the reproductive strategy of *F. vesiculosus*, which is indicated as broken arrows in the conceptual model (see Fig. 1).

To summarise we would like to stress the need to discuss and relate the effects of eutrophication both to adult and juvenile stages and to the two reproductive strategies of *F. vesiculosus*. To increase the success of management schemes and transplantations of *Fucus*, water quality, nutrient regimes, seasonal occurrence of filamentous algae and sediment, densities of grazers and the use of either summer- or autumn-reproducing individuals need to be taken into consideration.

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Can different vegetative states in shallow coastal bays of the Baltic Sea be linked to internal nutrient levels and external nutrient load?

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Key words: chlorophyll, filamentous algae, macrophytes, phytoplankton, water exchange time, phosphorus, coastal bays

Abstract

Two different vegetative states, i.e. one clear water state dominated by benthic macrophytes and one turbid state dominated by phytoplankton, are commonly found in shallow lakes. In this study we investigated vegetative states in shallow bays of the Baltic proper to see if different vegetative states could be defined. We aimed to couple different types of dominating vegetation to the external load of nutrients. We further investigated the external load of nutrients, the water exchange time and the nutrient level in the water and determined if they could be linked to the vegetative state of a shallow bay and used as management tools. Three vegetative states were found in the shallow (less than 3 m deep) and small bays (varying between 0.01 to 0.23 km^2) studied. These states were correlated both to the external load of phosphorus from the local watershed and to the total phosphorus (TP) concentrations in the water column. The first state (1) had clear water and low chlorophyll *a* concentrations (2–7 $\mu g\ l^{-1}$), relatively dense cover (20–36%) of rooted macrophytes and low TP concentrations (below 32 $\mu g\ P\ l^{-1}$). The second state (2) had very dense stands (45–103% cover) of rooted macrophytes covered by (39–68%) of filamentous green algae. In this state the water was clear, with low chlorophyll *a* (2–7 $\mu g\ l^{-1}$), intermediate TP concentrations in the water (42–45 $\mu g\ P\ l^{-1}$) and low TN (200–350 $\mu g\ N\ l^{-1}$) concentrations. In the third state (3), the unvegetated bottom areas were larger and chl *a* and TP levels in the water column were high, between 16 to 20 μg chl *a* l^{-1}, $> 45\ \mu g\ P\ l^{-1}$, respectively. One bay (bay 5) did not fit into any of these states and had high chl *a* and nitrogen concentrations (23 μg chl *a* l^{-1} and 1198 $\mu g\ N\ l^{-1}$) although the TP concentrations (34 $\mu g\ P\ l^{-1}$) and the external load of phosphorus were relatively low. This could be due to the long water exchange time of the bay or indicate alternative vegetative states within an interval of nutrient concentration. Based on our studies we propose that the point at which a bay shifts from one state to another depends on the levels of the external nutrient load, the water exchange time and the depth of the bay. We also suggest that the TP concentration in the water is the single best parameter to indicate the state of a bay. However, more data are needed before this conceptual model can be used as a tool in coastal zone management plans for shallow brackish bays.

Introduction

One of the objectives in coastal zone management is to understand the mechanisms by which both natural and anthropogenic factors maintain or alter structures and functions of shallow coastal benthic communities. A large number of factors are known to directly affect benthic vegetation in shallow coastal areas of the Baltic Sea, although the main factor causing changes was found to be nutrient enrichment (for reviews see Cederwall & Elmgren, 1990; Bonsdorff et al., 1997; Kautsky & Kautsky, 2000). During the last decades there has been an increasing number of observations of phytoplankton and filamentous algal blooms causing declines in the perennial benthic vegetation and the fauna in response to eutrophication, e.g. in Poland (Kruk-Dowgiallo, 1991), in Sweden, (Kautsky, et al., 1986; Pihl et al., 1995; 1999; Schramm, 1996; Eriksson et al., 1998) and in Finland (Norkko & Bonsdorff

1996; Bonsdorff et al., 1997; Bäck et al., 2000, Vahteri et al., 2000). Other factors, such as reduced light (e.g. Norin & Wærn, 1973) temperature (e.g. Cambridge et al., 1990), and high ammonium concentrations (Kautsky, 1982), salinity (e.g. Wallentinus, 1979) and weather-induced disturbances (e.g. Idestam-Almqvist, 1998), may also have negative effects on species distributions. Different toxic compounds, e.g. chlorinated compounds from pulp mill industries (Kautsky et al., 1988) and boat paints (Andersson & Kautsky, 1996; Lindblad et al. 1989) and physical disturbances such as boat traffic (Rönnberg, 1981) have also been shown to negatively affect benthic vegetation.

Rich benthic vegetation in shallow lakes plays a key role. For example, small lakes with a dense cover of benthic vegetation will have clear water (van den Berg et al., 1997) while lakes with reduced benthic vegetation will, according to Scheffer (1998), shift to a more turbid state. Similar shifts between different vegetative states have also been described in shallow coastal marine and estuarine areas (Duarte, 1995; Valiela et al., 1997) where a typical succession occurs along nutrient enrichment gradients from slow growing seagrasses and/or perennial macroalgal species, to filamentous or sheet-like, fast-growing macroalgae to phytoplankton blooms. The species composition may shift from less competitive, late-successional, thick leathery perennial species with a lower nutrient uptake capacity to fast-growing filamentous species (Breuer & Schramm, 1988; Kruk-Dowgiallo, 1991), or to sheet- or leaf-like algal species (Lavery et al., 1991; Sfriso & Marcomini, 1996). Such changes have been attributed to a higher surface: volume ratio in filamentous and sheet-like species compared to perennial species (Littler, 1980; Wallentinus, 1984; Duarte, 1995; Pedersen & Borum, 1997). However, a larger surface area to volume ratio cannot always be translated into greater nutrient uptake or growth capacity as shown by Lotze & Schramm (2000). Furthermore, the high tolerance of many filamentous algal species to changes in variables such as salinity and temperature enhances the success of these algae in eutrophic shallow bays and coastal areas of the Baltic, characterised by fluctuating environmental conditions (Wallentinus, 1979; Bonsdorff, 1992; Idestam-Almqvist 1998).

Due to the continuous land uplift along the Baltic Sea coast, shallow sheltered bays are slowly cut off from the sea resulting in a natural succession of plant species described by Münsterhjelm (1997). In the early succession, when the bay is still open to the sea, benthic vegetation is commonly dominated by the alga *Vaucheria* spp. and the eloides *Ceratophyllum demersum* L. and *Myriophyllum* spp. As the land uplift proceeds and the bay gets more enclosed, dense meadows of *Potamogeton pectinatus* L. and *Chara tomentosa* L. develop and may dominate the vegetation. At the last state of the succession, when the bay is connected to the sea only during high-water-level events, the annual species *Najas marina* L. and *Chara aspera* Deth. commonly dominate the vegetation. The species composition and density are, however, highly affected by major weather-induced disturbances as shown by Idestam-Almqvist (1998). At each of these successional states, an increased load of nutrients from the surrounding watershed may affect the vegetative states of the bay. At what nutrient threshold the vegetation shifts has, to our knowledge, not been investigated in the Baltic Sea. Thus, the aim of this study was to link different observed vegetative states to the external nutrient load and/or internal nutrient levels of a bay and to establish the nutrient threshold at which the vegetation in a bay may shift from one vegetative state to another.

Methods

The study areas

Ten small bays were studied from two different areas along the Swedish east coast, i.e. five from the northern Baltic proper (Stockholm archipelago) and five from the central Baltic proper (Bergkvara archipelago) (Table 1). The salinity in the investigated bays in the northern area have, during different visits varied between 4.7 to 7 psu while in the central Baltic proper bays the salinity varied between 6.2 to 7.7 psu. This variation in small, shallow bays with a long water exchange time is caused by a significant evaporation during dry periods and dilution during rainy periods, e.g. the salinity in bay 10, Pottan, has varied between 5 to 7 psu on different sampling occasions. The investigations were all performed in August when the vegetation is well developed. To check for weather-induced between-year variations in the vegetation, bays investigated in 1996, bay 9 and 5, were revisited in 1999 and 2000. Only the status of the vegetation was checked during the reinvestigations.

Water sampling and analysis

Water samples, for analyses of total phosphorus, total nitrogen and chlorophyll *a*, were collected only once

Table 1. Number, name, year of investigation for macrophyte cover and water chemistry, location and co-ordinates, bay area (km^2), depth (m), volume (m^3), water exchange time (w.e.t. in days), cover of benthic vegetation (%), cover of filamentous vegetation (%), cover of *C. demersum*, chl *a* ($\mu g\ l^{-1}$), TP = total phosphorus ($\mu g\ l^{-1}$), TN = total nitrogen ($\mu g\ l^{-1}$), external load of phosphorus ($\mu g\ P\ l^{-1}\ yr^{-1}$), external load of nitrogen ($\mu g\ N\ l^{-1}\ yr^{-1}$) in the 10 investigated bays.

Bay number and name	Field-study (Year)	Location	Lat.	Long.	Area (km^2)	Depth (m)	Volume (m^3)	w.e.t. (days)	Bent. veg. cover %	Fil. veg. cover %	C.d. cover %	Chl *a* ($\mu g\ l^{-1}$)	TP ($\mu g\ l^{-1}$)	TN ($\mu g\ l^{-1}$)	Ext. load P	Ext. load N
1, Östviken	2000	Bergkvara	562452	160619	0.03	2	19926	1.8	28.15	7.89	0.02	5	29	210	0.07	7.37
2, Appleviken	2000	Bergkvara	562415	160556	0.01	1.5	4846	0.5	22.19	11.76	0.05	2	30	250	0.02	1.38
3, Rumpsaxarviken	1999	Stockholm	590714	182020	0.05	1.1	20319	7.2	20.29	2.61	0.00	4	31	446	4.20	135.74
4, Slängen	1999	Stockholm	591415	183243	0.07	2.5	76830	17.6	35.81	0.00	0.00	7	32	683	1.01	66.16
5, Dyviksmaren	1996	Stockholm	591148	182417	0.05	2	38815	28.9	23.78	0.04	10.72	23	34	1198	14.40	288.85
6, Eneskärsviken	2000	Bergkvara	562355	160237	0.06	1.5	56489	12.1	56.48	38.91	0.41	3	42	200	26.12	324.18
7, Stengårdsviken	2000	Bergkvara	562354	160537	0.02	1.1	8238	14.3	102.62	68.49	0.00	7	43	290	35.78	3303.99
8, Kitteln	2000	Bergkvara	562347	160538	0.02	1	13806	16.7	45.58	60.42	0.05	2	45	360	32.77	318.23
9, Boviken	1996	Stockholm	585300	175223	0.23	2.2	204071	20.3	18.91	11.29	0.00	16	57	219	12.04	232.85
10, Pottan	1999	Stockholm	585126	175246	0.03	1.2	11936	32.8	6.69	3.12	27.67	20	89	327	616.07	11288.21

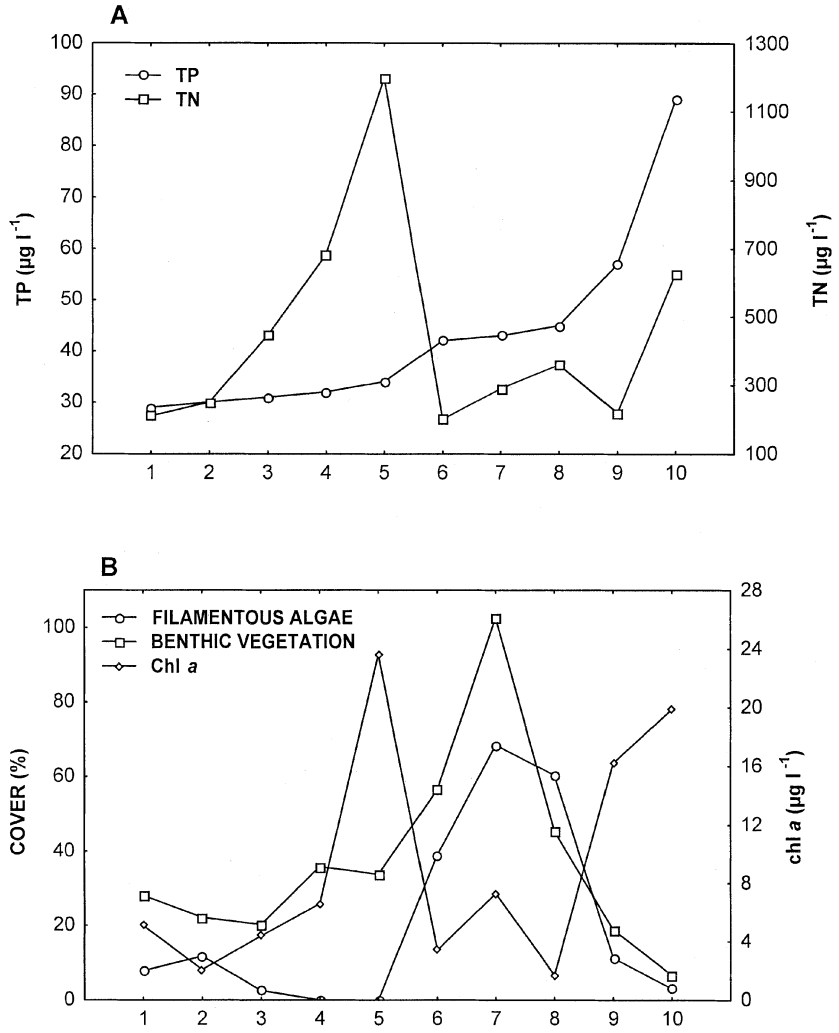

Figure 1. A. Cover of filamentous green algae and submerged benthic vegetation (%) and chlorophyll *a* concentrations (µg l^{-1}) in the ten studied shallow brackish bays. B. Total phosphorus (TP in µg l^{-1}) and total nitrogen (TN in µg l^{-1}) concentrations in bay numbers 1 to 10, sorted from the lowest to the highest concentration of TP. All data are from August.

at a depth of 0.5 to 1 m in the central part of the bay. To analyse chlorophyll *a* the water samples were immediately passed through GF/C filters and frozen until analysis. Chlorophyll *a* (chl *a* µg l^{-1}) was analysed using the method of Strickland & Parsons (1972). The samples was kept frozen ca. two weeks and not filtered before analysis. Water samples for total phosphorus (TP µg l^{-1}) and total nitrogen (TN µg l^{-1}) were analysed using the standard methods SS 02 81 27-2 and ASN-62-04/84, respectively.

External nutrients load and water exchange times

The load of nitrogen and phosphorus from the local watershed was calculated according to a model presented by Swedish Environmental Protection Agency (1996). The external load of phosphorus and nitrogen to the bays from the surrounding watersheds were significantly correlated ($p = 0.01$, $r = 0.98$), indicating a close link between the runoff of these two nutrients or an auto-correlation between the two parameters due to the calculation method. In this study, we have chosen to use only the external load of phosphorus. The model values are based on calculations of nutrient leakage from a set of different land ecosystems, i.e. forest, removed forest, arable land, bog, lake, 'other' land ecosystems and from households within the watershed. Air deposition is included in the formulas for the different land ecosystems. The leakage from forest

have been calculated according to following formula: TP (kg P ha^{-1} yr^{-1}) = 0.00014*Q-0.00383 where Q = yearly runoff in mm. In areas with removed forest the increased external load of phosphorus is estimated to be 200% during 3 years, compared to the external load of phosphorus from the forest. Ditching is approximated to increase the external load of phosphorus 3 times during 1 year. Of the removed forest, 0.1% was approximated to be ditched. Phosphorus is estimated to leak twice as much from bogs as from forests. Arable land was approximated to leak 0.6 kg P ha^{-1}. Wintergreen arable land is supposed to leak 75% less nitrogen than common arable land and equally much phophorus in the calculations. For arable land, 42.2% is estimated to be wintergreen. Total air deposition (both dry and wet) of phosphorus on open water, lakes and bays, is calculated to be approximately 8 kg P km^{-2} yr^{-1} (Swedish Environmental Protection Agency, 1996). Other land ecosystems are estimated to leak phosphorus similar to that of the forest.

Persons in households within the watersheds were estimated to produce 4.8 g N yr^{-1} and 0.9 kg P yr^{-1}. The number of persons living in the watershed was estimated to be 2.3 persons per household and the households used as summerhouses was estimated to be occupied 110 days per year. Households connected to wastewater treatment were not included in the calculations.

Data on average runoff of water from the watershed were taken from SMHI (1994) and varied between 5.5 l s^{-1} km^{-2} in the southern area and 7 to 8 l s^{-1} km^{-2} in the northern area. The total yearly amount of water from the watershed was calculated for each watershed. Data for the calculations of nutrient load and different type of land areas within the watersheds were measured from polygons on maps made in Ocad 7.0. The maps were vectorised from topographical maps, scale 1:12500.

The exchange time of the water in the bays was calculated according to the formula: ln Ty = -4.36 * $\sqrt{}$ (100*(bay opening area/bay surface area)) $+3.49$, where Ty = predicted water exchange time and -4.36 and $+3.49$ = empirical constants (Pilesjö et al., 1991). The predicted values are highly correlated to empirical values, r^2 = 0.93, according to Pilesjö et al. (1991). To obtain a value on yearly exchanged water mass, the water volume in the bays was calculated and multiplied by 365 days and divided by Ty. The bay opening area and the volume was calculated according to Håkanson et al. (1984). The calculation was based on measurements from maps in Ocad 7.0. Data for drawing the topographic maps were collected in field. Data on yearly external load of nitrogen (ton) and phosphorus (kg) from the external load model (Swedish Environmental Protection Agency, 1996), were recalculated to µg and divided with the yearly exchanges water mass, in litres, which gave the parameter external load of nutrient per exchanged water mass per year, in µg l^{-1} yr^{-1}.

Vegetation cover

The estimate of cover of the submersed vegetation and filamentous algae was performed by snorkelling along 1 or 2 transects, 2 m wide, placed perpendicular to the shoreline and extending from the shoreline to the deepest area of the bay. The transect, a sinking line with 1 m markers, was placed subjectively in the investigated bays. Each transect was divided into zones and within each zone the cover of species of rooted benthic vegetation, including Charophyceae and filamentous algae, were estimated in seven different classes; 0.1, 5, 10, 25, 50, 75 and 100% cover. The cover, as percentage of the transect area for the species was calculated as follows: Cover (in % of transect area) = $A_i B_i C_i$, where A = cover of the species in each zone, B = the zone's proportion of the transect and C = the transect's proportion of the total transect length of the bay. The cover of species was then summarised into the two plant groups, benthic macrophytes, including Charophyceae, and filamentous algae. The bottom area colonised by emergent vegetation was measured on maps and the vegetation cover in % of the bay area was multiplied with the open water part of the bay (total bay area – emergent vegetation area) to calculate plant group cover in % of bay area. The depth and point on the transect, where the vegetation ended, was noted and from that the unvegetated area was approximated on topographical maps using Ocad 7.0. The accuracy of the approximation was confirmed by snorkelling.

Results

The depth of the investigated bays varied between 1 to 2.5 m and the area between 0.01 to 0.23 km^2. Bays 1 and 2 had the shortest water exchange time (less than 2 days) and bays 5 and 10 the longest, (about 30 days; Table 2). TP in the water was positively correlated with external load of phosphorus (p = 0.005;

Table 2. Correlation matrix for the parameters: external load of phosphorus ($\mu g\ P\ l^{-1}\ yr^{-1}$), total phosphorus (TP in $\mu g\ l^{-1}$), total nitrogen (TN in $\mu g\ l^{-1}$), chl *a* ($\mu g\ l^{-1}$), cover of benthic vegetation (%), cover of filamentous vegetation (%) and water exchange time (w.e.t., in days).

Parameter	Ext. load P ($\mu g\ l^{-1}\ yr^{-1}$)	TP ($\mu g\ l^{-1}$)	TN ($\mu g\ l^{-1}$)	Chl *a* ($\mu g\ l^{-1}$)	Bent. veg. cover %	Fil. veg. cover %	w.e.t. (days)	Transformation
Ext. load P	1.0							ln
TP	0.76	1.00						sqrt
TN	0.12	−0.24	1.00					sqrt
Chl *a*	0.48	0.52	0.47	1.00				sqrt
Bent. veg. cover	0.08	−0.34	−0.03	−0.35	1.00			sqrt
Fil. veg. cover	0.23	0.11	−0.57	−0.51	0.68	1.00		sqrt
w.e.t	0.80	0.70	0.49	0.81	−0.19	0.23	1.00	–

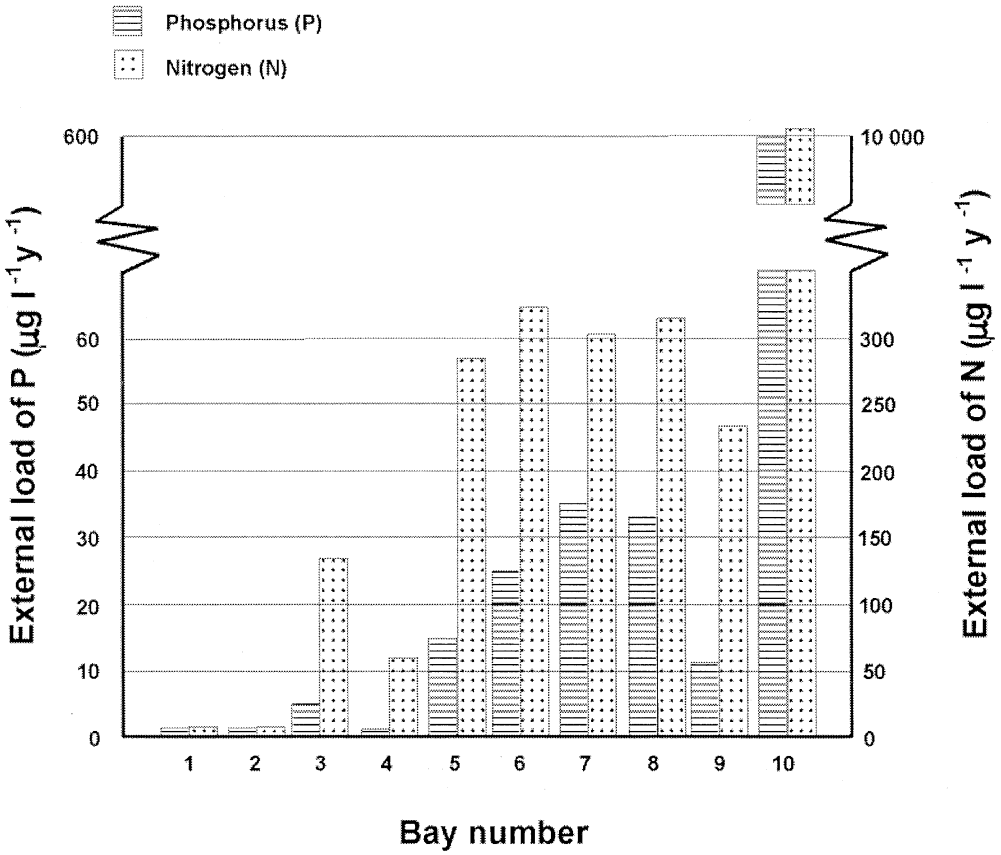

Figure 2. The external load of total nitrogen and phosphorus ($\mu g\ l^{-1}\ yr^{-1}$), calculated as external load of nutrients per year per exchanged amounts of water per year. External load was calculated according to Swedish Environmental Protection Agency (1996) and the water exchange time (days) according to Pilesjö et al. (1991).

$r = 0.80$) (Table 2). Based on this correlation the bays were ranked after TP concentration, from lowest (bay 1) to the highest (bay 10) (Fig. 1). The water exchange time was also significantly correlated to the external load of phosphorus ($p = 0.02$; $r = 0.70$), with the lowest external load of phosphorus in bay 2 and the highest load in bay 10. The correlation is due to the calculation method. Chlorophyll *a* was positively correlated with increasing water exchange time ($p = 0.004$; $r = 0.81$) but not with TP or TN in

the total dataset (Table 2). However, chlorophyll *a* was found to increase with increasing concentrations of TP both below 32 μg P l^{-1} (bay 1–4) and at high TP levels (bay 9 and 10) (Fig. 1a,b) and the increasing concentrations of TP and TN is partly due to the increasing concentrations of chl *a*, i.e. to the large amount of phytoplankton in the water. Both TP and chl *a*were positively correlated to the water exchange time ($p = 0.02$; $r = 0.70$, $p = 0.004$; $r = 0.81$). At TP concentrations above 45 μg P l^{-1}, chl *a* concentrations were very high (bays 9 and 10). At intermediate TP concentrations (42–45 μg P l^{-1}), i.e. bays 6, 7 and 8, chl *a* concentrations were instead low and coincided with a high cover of both benthic vegetation and filamentous green algae (Fig. 1a,b). The highest concentrations of chl *a* occurred in bays 5, 9 and 10. These bays also had the longest water exchange times of all bays (Table 1) and were the only ones with unvegetated bottom areas.

The cover of rooted benthic vegetation and filamentous algae (mainly *Cladophora glomerata* L. Kütz) was high in some of the bays with intermediate external nutrient load, i.e. bays 6 to 8 (Fig. 2). At high external loads of nutrients (bay 10), the cover of rooted benthic vegetation was low with large unvegetated areas and a high abundance of the free-floating macrophyte *Ceratophyllum demersum* combined with a high chl *a* concentration and low abundance of filamentous algae. The rooted macrophytes, *Myriophyllum spicatum* L., *Potamogeton pectinatus* and *Najas marina*, occurred in most of the investigated bays. *Ruppia spp.* and *Fucus vesiculosus* L., growing attached to small stones or rocks, were also noted in some bays (1, 2, 6, 7 and 8). The dominating filamentous algal species was *C. glomerata* but *Spirogyra* spp. also occurred growing intermingled with the macrophytes. In bays 5 and 10, free-floating *C. demersum* was common (Table 1). Several stonewort species were found in the studied bays. The red-listed *Chara horrida* Wahlstedt was only found in bay 8 (Gärdenfors, 2000). Other stoneworts found were *Chara tomentosa* L., in bays 3, 4, 5 and 9, *Chara baltica* Bruz., in bays 4, 5, 6 and 8, and *Chara canescens* Desv. & Lois., in bays 3 and 4. The common stonewort *Chara aspera* was found in bays 1, 2 and 4.

We defined three vegetative states in the studied bays. The first state (1) had clear water and low chlorophyll concentrations (2–7 μg l^{-1}), relatively dense cover of rooted macrophytes (20–36%), and low TP concentrations (below 32 μg P l^{-1}). The second state (2) had very dense stands of rooted macrophytes, between (45–100%), covered by filamentous green algae (39–68%). In this state the water was clear, with low chlorophyll *a* (2–7 μg l^{-1}), intermediate TP concentrations (42–45 μg P l^{-1}) and low TN (200–360 μg N l^{-1}) concentrations. In the third state (3), the unvegetated bottom areas were larger and chl *a* and TP levels in the water column were high, between 16 to 20 μg chl *a* l^{-1}, >45 μg P l^{-1}, respectively. During the field investigation the open water outside the bays was clear and showed no signs of microalgal blooms. Therefore, the observed blooms of phytoplankton, within the bays, seem, not to be coupled to blooms of *Nodularia spumigena*, *Aphanizomenon* spp. or other cyanobacteria or microalgae in the open sea. Bays defined as state 1, i.e. bays 1–4, had the lowest external nutrient load, < 4.2 μg P l^{-1} and <136 μg N l^{-1}, respectively. Bays defined as state 2, i.e. bays 6 to 8, had much higher external nutrient loads (Table 1, Fig. 2), between 26 to 36 μg P l^{-1} and 318 to 3304 μg N l^{-1}. Bays defined as state 3 had both higher, bay 10, and lower, bay 9, external nutrient load concentrations then bays defined as state 2. Both bay 5 and bay 9 have longer water exchange times and are slightly deeper then bays 6 to 8, which may be the reason for the difference in chl *a* and TP concentrations between bays 6 to 8 and bays 9 and 10.

Discussion

Both short- and long-term changes in benthic vegetation caused by increased nutrient inputs to the coastal waters have been well documented (e.g. Kautsky et al., 1986; Norkko & Bonsdorff, 1996, Pihl et al., 1995, 1999; Bonsdorff et al., 1997; Eriksson et al., 1998; Bäck et al. 2000, Vahteri et al., 2000). In this study we indicate that different vegetative states may be linked to a specific TP level in shallow coastal bays of the Baltic proper and that the TP concentrations in the water can be correlated to the external nutrient load. Further we suggest that a specific threshold TP level, between 30 to 35, when the vegetation shifts from relatively dense rooted benthic plant communities with clear water to communities with very dense meadows of rooted submerged vegetation covered by filamentous algae or to a state with turbid water and phytoplankton blooms. The bays with TP concentrations below 32 μg l^{-1} also had the lowest external load of nutrients, <5 μg P $l^{-1}y^{-1}$ and <137 μg N l^{-1} yr^{-1}, respectively, which could be used as threshold value for the external load of phosphorus.

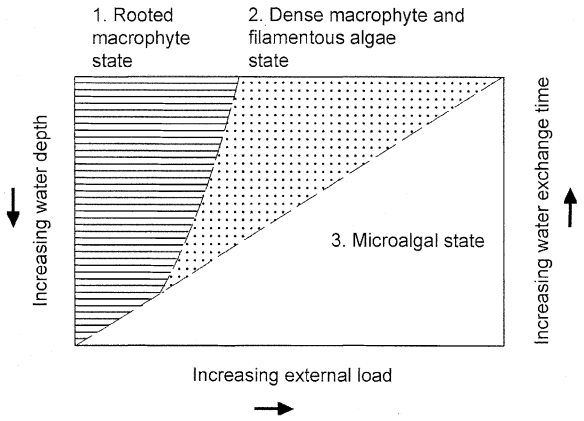
1. Rooted macrophyte state
2. Dense macrophyte and filamentous algae state
3. Microalgal state
Increasing water depth
Increasing water exchange time
Increasing external load

Figure 3. A conceptual model of the links between three vegetative states and increasing external load of nutrients and water exchange time in shallow brackish bays of the Baltic Sea. (1) Rooted macrophyte state, (2) Macrophyte/filamentous algal state, (3) Microalgal state.

Submerged rooted macrophyte species have been shown to have a competitive advantage over macroalgae and phytoplankton in nutrient-poor environments due to their ability to take up nutrients from the sediment (Barko et al., 1991, and references therein). Increased nutrient load from the surrounding area may result in a progressive replacement of these species to fast-growing filamentous algae and phytoplankton (e.g. Blindow, 1991). Valiela et al. (1997) have suggested that the nutrient levels at which a bay will change from one state to another depend on the water exchange time because of a higher dilution of sea water in bays that have short exchange times. Similarly, in the 10 investigated bays we observed a difference between plant group abundance in the shallower bays compared to the deeper bays and between bays with different water exchange times and different amounts of external load of nutrients. In Fig. 3 we present a hypothetical model describing the relationship between the occurrences of the three different vegetative states, external nutrient load and water exchange time, respectively. The model predicts, based on the bays investigated in the Baltic proper, that at low levels of external nutrient loads, shallow coastal bays will be in state 1, i.e. the rooted macrophyte state and have a relatively dense cover of benthic vegetation. Above a given threshold of external nutrient load, depending on the water exchange time and the depth, the bay will shift from state 1 to state 2 or 3, with extremely dense cover of benthic vegetation and filamentous algae or phytoplankton blooms. When the shading from filamentous algae in state 2 gets too high (Fig. 3) the benthic vegetation will collapse causing a shift from state 2 to state 3, the microalgal state. In bays shallower then 1.5 m (e.g. bays 6–8), light rarely limits rooted benthic vegetation, and shading by phytoplankton would need to reach extreme levels to be able to out-compete the rooted vegetation. We predict such bays to seldom reach state 3 but instead have dense filamentous algae covering the benthic vegetation at high external nutrient loads. Contrary, in slightly deeper bays, the benthic vegetation may become light-limited and may therefore shift to state 3, at a lower external nutrient load then the shallower bays.

To our knowledge this is the first time that nutrient thresholds of internal TP concentrations and external nutrient loads have been proposed to predict the vegetative state of shallow brackish bays. The high TP concentration in some of the bays, i.e. bays 9 and 10, may be explained by large blooms of phytoplankton, while in some of the other bays it is probably explained by internal load from the sediment, i.e. bays 6 to 8, or by a combination of those and the external load of phosphorus. The TP concentration within the water is therefore not an independent threshold parameter but a parameter that is suggested to best indicate the state of the bay.

The conceptual model for shallow brackish bays is supported by other studies (Sfriso et al., 1987; Lavery et al., 1991; Nienhuis, 1992; Scheffer et al., 1993) indicating that changes in vegetation occur rapidly and not as a gradual change in the abundance of different plant species. For example, Lavery et al. (1991) showed that the occurrence of a macroalgal bloom was a sudden event and appeared to have resulted from nutrient availability surpassing a threshold. Bays with larger openings to the sea and/or intense boat traffic may temporarily have high concentrations of re-suspended sediments in the water, due to a high extent of wind and wave action and/or propeller action, which may reduce light penetration. In bay 9, for instance, the large unvegetated area may be due to re-suspension of sediment caused by intense boat traffic, together with high chl *a* concentrations, which negatively affect the growth of benthic vegetation. Regional differences and difference in salinity (Wallentinus, 1979) may also affect at what nutrient load a bay shifts from one state to another state. For example, the freshwater species *Chara tomentosa* may not be able to form meadows and survive in bays in the Bergkvara archipelago, southern Kalmar Sound where the salinity often exceeds 7 psu. Differences in plant morphology may also be important when shifts between

vegetative states occurs, e.g. species with their meristem close to the sediment surface are expected to be out-competed by species with apical meristem or free-floating species in nutrient-rich turbid waters (Duarte, 1995). Contrarily, at low nutrient levels, rooted submerged macrophyte species have been shown to be competitively superior, since they can extract nutrients from the sediment. Finally, Körner & Nicklisch (2002, and references therein) have shown that submerged macrophyte species, e.g. Myriophyllum spicatum and Ceratophyllum demersum can inhibit growth of selected phytoplankton species even if the nutrient levels never reach limiting concentrations. Thus, also allelopathic interactions may be involved in the shift between vegetative states although further in situ studies will be needed to gain knowledge on the ecological relevance of such substances (Körner & Nicklisch, 2002). However, sediment factors may interact with above-sediment conditions, such as light availability and wave exposure, resulting in changes in growth and morphology (Idestam-Almqvist & Kautsky, 1995), which in turn may affect the survival of and competition between different species.

This study is a first attempt to set nutrient threshold limits critical to sustain specific benthic vegetation in shallow coastal bays of the Baltic Sea. Total phosphorus concentrations in the water mass were found to be the best single parameter explaining the state of the bay together with the external nutrient load and the water exchange time. We further present a conceptual model relating external nutrient load, depth and water exchange time to the vegetative state of a bay. However, large differences are expected to occur between bays located in the inner or outer archipelago, as well as between regions in the Baltic Sea. Inter- and inner-annual variations are also to be expected. Thus, further investigations are urgently needed to verify the generality of the proposed critical nutrient limits before they can be used in predictive models as tools in local planning and management decisions.

Acknowledgement

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Factors controlling long-term changes of the eutrophicated ecosystem of Pärnu Bay, Gulf of Riga

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Key words: Baltic Sea, long-term changes, phytoplankton, zooplankton, *Mysidacea*, fish larvae

Abstract

Phytoplankton, mesozooplankton, mysids and fish larvae were studied during 15–29 annual cycles measured weekly to monthly in Pärnu Bay, the Gulf of Riga. The monthly variability of the biological data was related to temperature, ice conditions, salinity, influx of nutrients, the North Atlantic Oscillation (NAO) index, cloudiness and solar activity. Phytoplankton development was mainly a function of the NAO index. For the whole study period the abundance of zooplankton increased with increasing water temperature and solar activity. Significant correlations between phytoplankton and zooplankton densities were found until 1990. After the invasion of the predatory cladoceran *Cercopagis pengoi* in 1991, the zooplankton community was likely to be regulated by the introduced species rather than phytoplankton dynamics. The increased abundances of rotifers and copepods triggered the increase in mysid densities. The development of herring larvae was positively affected by the high density of copepods and rotifers but also by increased eutrophication. Until 1990 there was no significant relationship between the density of zooplankton and herring larvae. A negative relationship between the density of zooplankton and herring larvae in the 1990s suggests that the major shift in zooplankton community resulted in food limitation for herring larvae. The results indicated that (1) atmospheric processes in the northern Atlantic explain a large part of the interannual variation of the local phytoplankton stock, (2) trophic interactions control the development of pelagic communities at higher trophic levels, and (3) the introduction of an effective intermediate predator has repercussions for the whole pelagic food web in Pärnu Bay.

Introduction

In recent decades the pelagic communities of the Baltic Sea have substantially changed (Elmgren, 1989, 2001). These changes have been attributed either to shifts in climate (Hänninen et al., 2000; Möllmann et al., 2000; Dippner et al., 2001; Kornilovs et al., 2001), to trophic status (Wulff et al., 1986; Heerkloss et al., 1991; Elmgren, 2001) or to both (Elmgren, 1989; Ojaveer et al., 1999), but also to top-down regulation (Rudstam et al., 1994). The prevalence of these processes depends highly on the region considered and the debate about the significance of human activity in these changes remains.

Besides climatic changes and eutrophication, biological invasions are observed at an increasing rate in the Baltic Sea (e.g. Leppäkoski & Olenin, 2001). The introductions of benthic invertebrates have resulted in large scale ecological changes and economic damage (Kotta, 2000; Kotta et al., 2001; Leppäkoski & Olenin, 2001). There has been much discussion about the pelagic invaders (Ojaveer & Lumberg, 1995; Ojaveer et al., 1998, 1999; Leppäkoski & Olenin, 2001). Besides increasing dominance in the communities, however, little evidence has been documented so far about the effects of introduced species on the pelagic food web.

The objective of this study was to investigate the effect of local environmental variables and large scale climatic patterns on the interannual variability of the pelagic ecosystem in Pärnu Bay, Gulf of Riga. Phytoplankton, mesozooplankton, mysids and fish larvae were studied during 15–29 annual cycles measured weekly to monthly. The extensive dataset enabled us

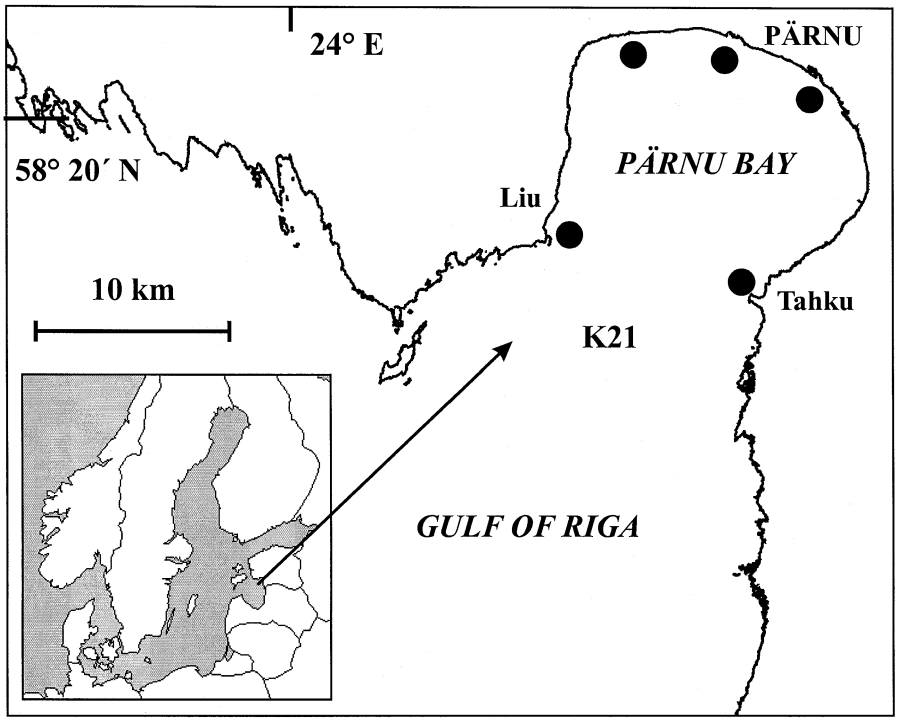

Figure 1. Map showing the study area. K21 represents the sampling station for hydrography, phytoplankton, zooplankton and mysids. Bullets represent ichtyoplankton sampling sites.

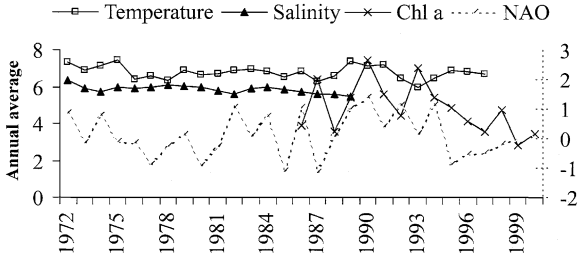

Figure 2. Interannual changes in water temperature (^{o}C), salinity (PSU), chlorophyll *a* concentration (μg l^{-1}) in Pärnu Bay and the NAO index.

to estimate if bottom-up or top-down effects dominate in the pelagic food web and whether changes in external nutrient loads and/or biological invasions have substantial effects on the different trophic levels of the system.

Material and methods

Pärnu Bay is a shallow semi-enclosed water basin in the NE Gulf of Riga, Baltic Sea (Fig. 1). The surface area of the bay is about 700 km^2 and the volume is about 2 km^3. Its maximum depth gradually increases from 7.5 m in the inner part (NE of the Liu–Tahku line) to 23 m in the SW part. The hydrological conditions of the bay are formed under the complex influence of meteorological processes, river discharge (Pärnu River, freshwater inflow 2 km^3 annually), and water exchange with the open part of the Gulf of Riga. The currents are generally weak in the area and are mainly wind-induced. The morphology of the coastline is relatively simple and consists mainly of sandy beaches and extensive reed stands. Prevailing sediments are sand or silty sand.

Pärnu Bay is suffering from a heavy anthropogenic eutrophication. The town of Pärnu, with its 70 000 inhabitants, and the Pärnu River are the major sources of eutrophication in the bay. Previously, the bay was contaminated with toxic substances and nutrients due to insufficient purification of sewage and industrial wastewater. As a result, the concentrations of total nitrogen (totN), total phosphorus (totP) and silicate (Si) increased on average by a factor two in the seawater whereas primary production of phytoplankton substantially increased in the 1970s and 1980s (Ojaveer, 1995; Tenson, 1995). Since 1990, the wastewater of Pärnu town is mechanically and biologically treated, but some minor outlets are still operating without

Table 1. Studied parameters

Parameter	Years	Months	Sampling frequency
Temperature	1972–2000	April–November	Weekly (daily)
Salinity	1972–2000	April–November	Weekly (daily)
Phytoplankton (Chl *a*)	1986–2000	April–November	Weekly
Zooplankton	1972–2000	April–November	Weekly
Mysids	1974–1997	April–November	Fortnightly
Herring larvae	1974–1997[a]	May–July	Weekly

[a]Data for 1981, 1985, 1990 are missing and the larvae are not divided into different length classes in 1988, 1991 and 1997.

wastewater treatment. However, a significant source of nutrients is Pärnu River, which constitutes about 10% of total riverine runoff to the Gulf of Riga and annually brings about 40–50 t totP and more than 4000 t totN into Pärnu Bay (Suursaar, 1995).

Data on studied parameters were collected over different time periods and with different frequencies (Table 1). Sampling was performed at a 10 m station (K21) except for fish larvae, which were caught in five spawning grounds around the coast of Pärnu Bay. Chlorophyll *a* (Chl *a*) was measured in discrete samples at 1, 5 and 10 m of water depth and the obtained values were averaged. For measuring Chl *a* concentrations, one liter of water was filtered through glassfibre filters and extracted for 24 h in darkness at 4–6 °C with acetone. Absorbance of the extract was measured spectrophotometrically (Yanaco UO-2000, Secomam S250I and Hitachi) and Chl *a* concentration calculated according to Jeffrey and Humphrey (1975). Zooplankton samples were collected by vertical hauls through the whole water column with a Juday net (mouth surface area 0.1 m^2, mesh size 90 μm). Samples were preserved in 4% formaldehyde solution and analysed by a routine method suggested by the Baltic Monitoring Programme (HELCOM, 1988). Copepods were identified to species with development stage levels and nauplii as separate groups. A modified Rass dredge was used for the sampling of mysids (mouth surface area 0.12 m^2, mesh size 400 μm, filtrated water volume 17 ± 3 m^3). All samples were stored in 4% formaldehyde solution. The mysids were determined to species level (Kotta & Kotta, 1999). Ichtyoplankton was sampled by towing a Hensen net (mouth diameter 80 cm, mesh size 500 μm) in a 1–2 m deep surface layer. Towing speed was 3 knots. Herring larvae were reported for a 10 min catch (ind. $catch^{-1}$). The larvae were divided into three size classes: shorter than 10 mm, between 10 and 20 mm and longer than 20 mm.

During sampling, temperature in the surface and bottom water was measured. Temperatures measured six times daily at the Kihnu and Pärnu hydrometeorological stations were related to our measurements (nonlinear regressions, $r^2 > 0.95$). These relationships were used to calculate water temperatures on a daily basis. Data on salinity, freshwater inflow from the Pärnu River, ice conditions and cloudiness were obtained from the Estonian Hydrometeorological Institute. The freshwater inflow of Pärnu River was used as an estimate of nutrient loads into Pärnu Bay. The Wolff number was used as a proxy of solar activity. As a proxy of atmospheric behaviour the index of the North Atlantic Oscillation (NAO) was used (Barnston & Livezey, 1987; Ottersen et al., 2001). The NAO is an alternation in the pressure difference between the subtropic atmosphere high-pressure zone centered over the Azores and the atmospheric low-pressure zone over Iceland. The NAO is primarily a winter phenomenon as its correlations with wind, temperature and precipitation are strongest during winter. The link between the NAO and seawater temperature may persist over the summer, however, being highly region dependent and should be assessed for each site separately (e.g. Ottersen et al., 2001). During years with high NAO there is a substantial increase in rainfall and consequently the freshwater inflow into the Baltic Sea is higher (Hänninen et al., 2000). Increased pressure differences result in stronger winter storms and higher winter temperatures in the northern Europe (Rogers, 1984). Average NAO values and three different NAO indices (NAO December through February, NAO December through March, NAO in March) were used to relate the large-scale climate pattern to the variation of biological data in the study area.

All studied time series were transformed to monthly means. Missing values in the time series of the biological data were replaced by averages of neighbouring values. These replacements constituted less than 5–10% of the whole dataset. Seasonality and autocorrelation were removed from the time series (Chatfield, 1984; Viitasalo et al., 1995). Remaining residuals were considered to represent the numerical response of each series to the surrounding environment. Correlations and linear regression analyses were performed to describe relationships between abiotic and biotic data. Statistical significance was analysed for all results.

Results

Average water temperature varied considerably in Pärnu Bay over the period analysed. Substantially higher temperatures were measured in the beginning of the 1970s and the 1990s. Average salinity gradually decreased from 6.3 (1972) to 5.5 (1989) PSU. Unfortunately we do not have salinity data for the 1990s. However, other studies imply a salinity increase during last decade (Ojaveer et al., 1999). The NAO values were highly variable with no clear long-term trend (Fig. 2).

Average Chl *a* concentrations were relatively high with maximum values at 20 $\mu g\ l^{-1}$ during the phytoplankton spring bloom. Chl *a* concentrations increased moderately in the late-1980s and declined in the 1990s (Fig. 2).

Mean zooplankton abundances varied considerably from year to year (Fig. 3). Copepods and rotifers dominated the zooplankton community. Relatively high copepod abundances were observed in the beginning of the time series, followed by a decline in the mid-1980s. The values peaked in the late-1990s at 50.000 individuals m^{-3}. The dominant copepod species were *Acartia bifilosa* and *Eurytemora affinis*. Other species contributed by only 0.33% to the total copepod abundance.

In general, the dynamics of rotifers followed the trend of total zooplankton abundance. *Synchaeta* spp. and *Keratella* spp. were the dominating rotifers. Other species contributed by only 0.22% to the total rotifer abundance.

Cladoceran abundance was low in the beginning of the study period, increased twofold in the 1980s and dropped sharply in the beginning of the 1990s. Among the cladocerans, *Bosmina coregoni maritima* prevailed

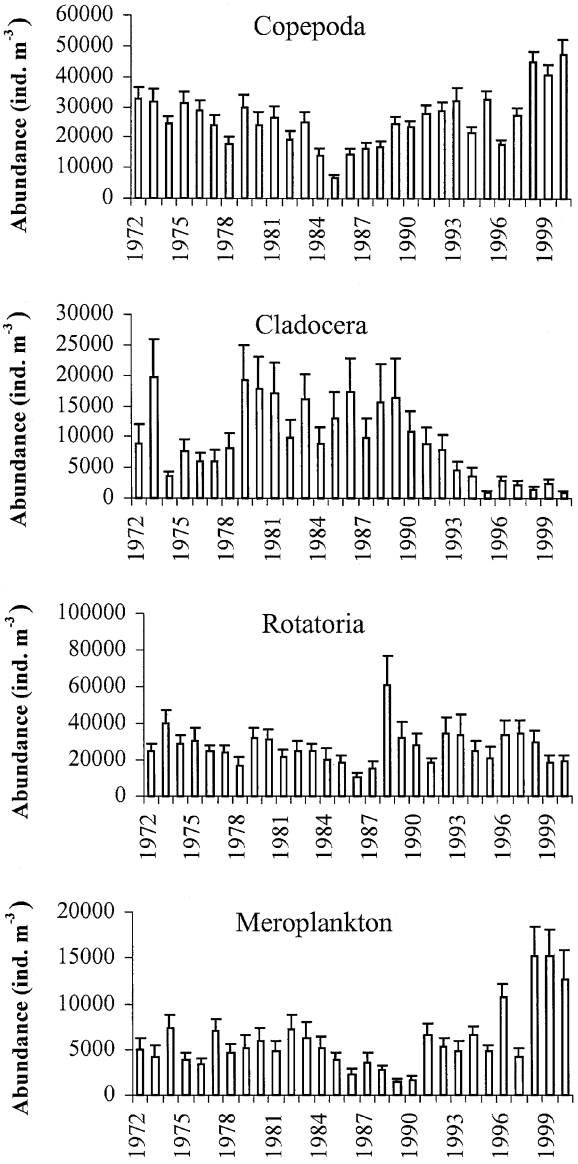

Figure 3. Interannual changes in the abundance (individuals m^{-3} ± S.E.) of different taxonomic groups of zooplankton in Pärnu Bay.

in the study area. Other species contributed by 17% to the total cladoceran abundance. In 1991, the predatory cladoceran *Cercopagis pengoi* (Rivier, 1998) was discovered in Pärnu Bay. Since then the population densities of this species have gradually increased. The maximum density of *C. pengoi* was estimated to 590 ind. m^{-3} in 2000.

Generally, meroplankton abundances were low and highly variable. Since the 1990s the contribution of meroplankton among other zooplankton taxa has significantly increased. In individual samples, mero-

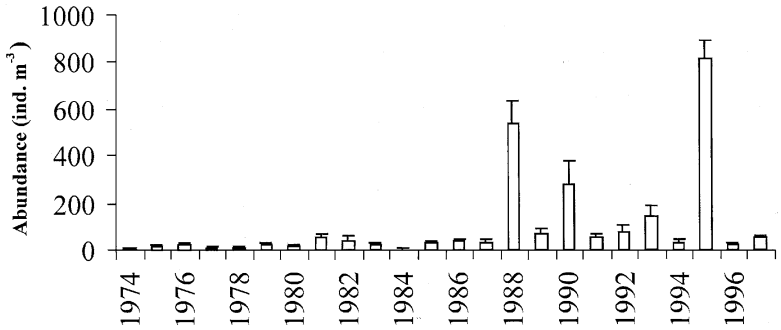

Figure 4. Interannual changes in the abundance (individuals $m^{-3} \pm$ S.E.) of *Neomysis integer* in Pärnu Bay.

plankton (primarily the larvae of the introduced polychaete *Marenzelleria viridis*) contributed by up to 83% to the total zooplankton abundance of 88 000 ind. m^{-3}.

The population dynamics of mysids had two distinct phases: low and relatively stable abundances from the early-1970s to the late-1980s, and high and fluctuating abundances from the late-1980s onwards (Fig. 4). *Neomysis integer* was practically the only mysid species found in Pärnu Bay. *Mysis mixta* contributed by only 0.06% to the total mysid abundance.

The abundance of herring larvae was highly variable and no clear trends could be identified. Larvae smaller than 20 mm were dominating in the samples (Fig. 5).

The variables best explaining the changes in pelagic communities are presented in Table 2. The annual average of the NAO index was a poorer predictor of interannual variability of biological data than the various NAO seasonal indices ($NAO_{Dec-Feb}$, $NAO_{Dec-Mar}$, NAO_{Mar}). The NAO seasonal (winter) indices reflected the severity of winter in the study area. The period of ice coverage and the sum of negative winter air temperatures were a linear function of the NAO seasonal indices ($r = 0.50$ and $r = 0.71$, respectively; $p < 0.01$). The $NAO_{Dec-Mar}$ index was linearly related to the light regime i.e. cloudiness ($r = 0.63$, $p < 0.001$). We found no significant correlations between NAO, riverine runoff and salinity in the study area ($p > 0.05$).

The NAO was a better predictor of average Chl *a* than the local environmental variables. Both annual average and maximum Chl *a* values were mainly a function of the NAO seasonal indices. With higher NAO values in winter, the average annual Chl *a* were higher. Higher values of maximum Chl *a* were observed after lower NAO values in March.

Depending on the taxonomic group considered, zooplankton abundances were related either to water temperature and/or solar activity. In general, the coefficients of determination of the models were low. The inclusion of Chl *a* did not improve the models of zooplankton dynamics. Total zooplankton abundance was best described by surface temperature, temperature gradient (i.e. temperature difference between surface and bottom layers) and solar activity. The abundance of copepods was mostly related to the temperature gradient ($r = 0.49$, $p < 0.01$), rotifers to maximum surface temperature ($r = 0.53$, $p < 0.05$), cladocerans to solar activity ($r = 0.41$, $p < 0.05$) and meroplankton to the duration of ice coverage ($r = 0.52$, $p < 0.05$), respectively.

During the study period there was a clear shift in the correlation between the densities of phytoplankton and zooplankton. Before the 1990s, the density of copepods was highly correlated to Chl *a* concentrations. However, the period of observation was short (i.e. five years) because Chl *a* sampling was carried out only since 1986. Since 1991, there was no correlation anymore ($p > 0.05$). The major change in zooplankton community structure was the invasion of the predatory cladoceran *Cercopagis pengoi* into Pärnu Bay in 1991. simultaneously with the increase in the density of *C. pengoi*, other cladocerans declined and copepods increased (Fig. 6).

Zooplankton density and winter severity were the best predictors of mysid abundance and biomass. Mysid abundance was a function of the density of rotifers and copepods. Mysid biomass was related to the density of copepods (i.e. *Eurytermora affinis*) ($r = 0.55$, $p < 0.01$) and the duration of ice cover ($r = -0.63$; $p < 0.001$). Since 1991, the maximum density of mysids declined with the concurrent invasion of *C. pengoi* ($r = -0.78$, $p < 0.001$). However, this relationship may partly involve the decline of Chl *a* in Pärnu Bay since 1994 ($r = 0.52$, $p < 0.05$).

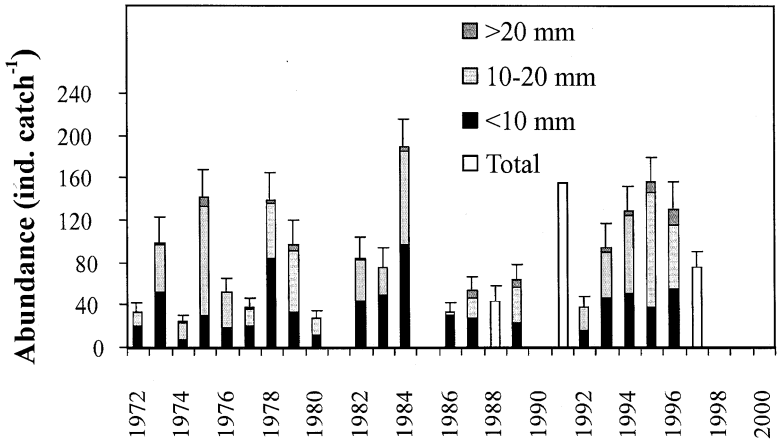

Figure 5. Interannual changes in the abundance (individuals catch^{-1} ± S.E.) of herring larvae in Pärnu Bay. Three size classes are reported. Data for 1981, 1985 and 1990 are missing and the division into length classes was not made in 1988, 1991 and 1997.

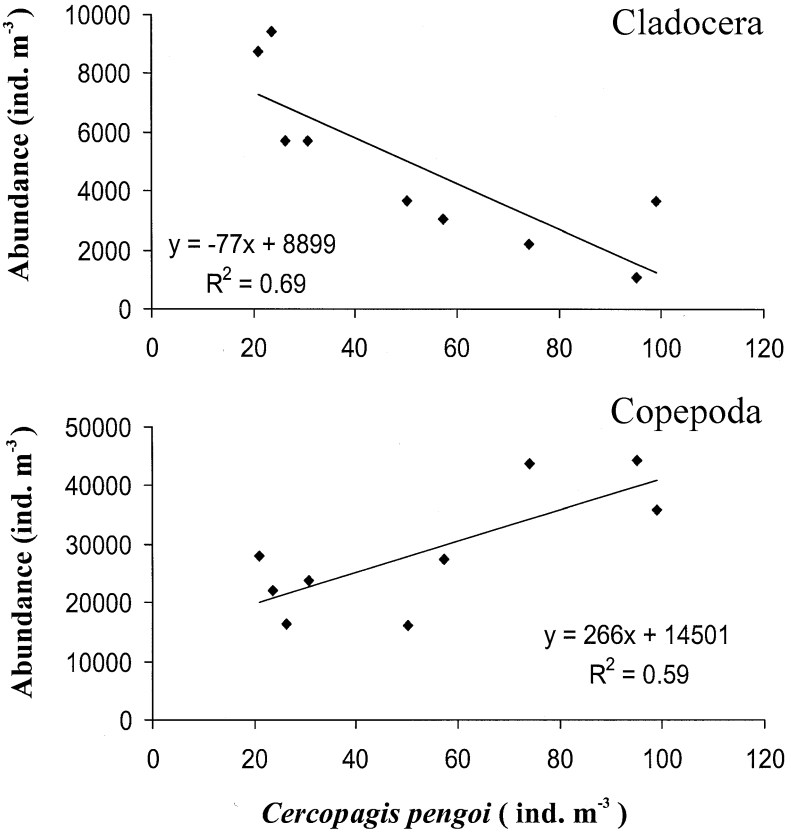

Figure 6. Relationship between the average yearly abundance of the predatory cladoceran *Cercopagis pengoi* and other cladoceran and copepods. The regressions and coefficients of determination are presented.

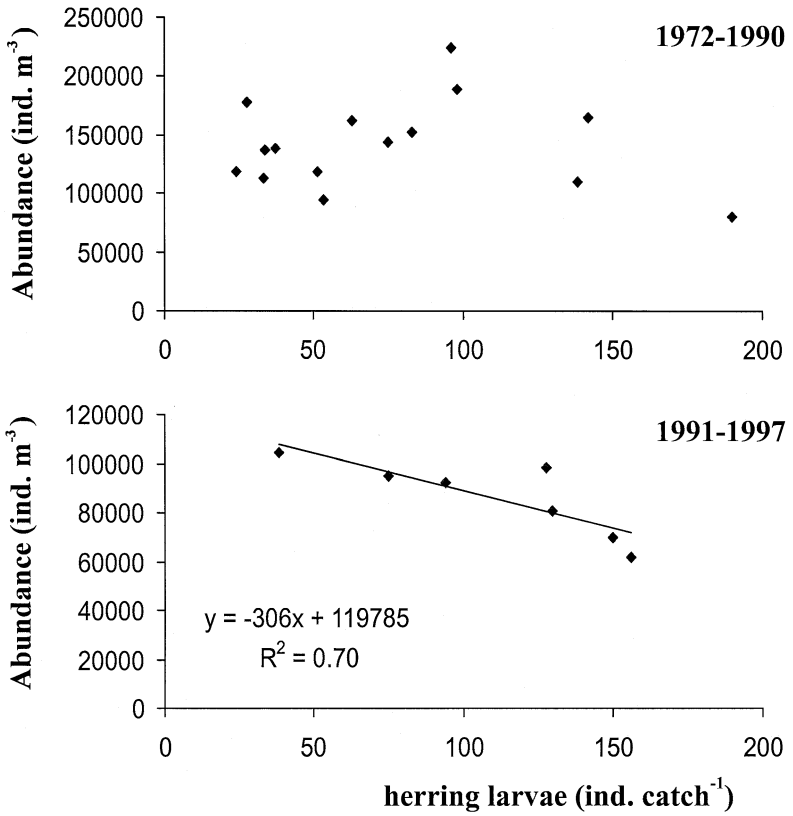

Figure 7. Relationship between the average abundance of herring larvae and zooplankton in July. The regression and coefficient of determination are presented.

Table 2. Results of linear regression analyses between studied variables. Prior to analysis, seasonality and autocorrelation was removed from all time series.

Dependent variable	Best independent variables (model)	*r* (model)	*p*
Chlorophyll *a* ($\mu g\ l^{-1}$)	$NAO_{Dec-Feb}$	0.80	<0.001
Maximum chlorophyll *a* ($\mu g\ l^{-1}$)	Maximum ice thickness (cm)	0.79	<0.001
Zooplankton abundance (ind. m^{-3})	NAO_{Mar} $(t^{o}_{surface} - t^{o}_{bottom})^2$ Wolff number	0.70	0.04
Copepods abundance in 1986–1990 (ind. m^{-3})	Max chl *a*	0.94	<0.001
Mysid abundance (ind. m^{-3})	Max abundance of *A. bifilosa* 3rd copepodite stage Abundance of *E. affinis* 3rd copepodite stage Max abundance of rotifers	0.67	<0.001
Herring larvae <10 mm (ind. $catch^{-1}$)	Runoff from the Pärnu River ($m^3\ s^{-1}$)	0.54	0.045
Herring larvae 10–20 mm (ind. $catch^{-1}$)	Duration of ice cover ($d\ yr^{-1}$) Max abundance of *Keratella* spp. Abundance of *A. bifilosa* 3rd copepodite stage	0.60	0.04
Herring larvae >20 mm (ind. $catch^{-1}$)	Max abundance of rotifers	0.57	<0.001

The density of herring larvae shorter than 10 mm was correlated with the average runoff from the Pärnu River. The density of the larvae between 10 and 20 mm was linked to the duration of ice cover and the density of *Keratella* spp. and *Acartia bifilosa*. The dynamics of herring larvae longer than 20 mm was related to the density of rotifers.

We found no significant correlations ($p > 0.05$) between the number of herring larvae and the zooplankton stock size in July before 1991. However, since 1991 a highly significant negative correlation between the density of herring larvae and zooplankton was observed ($r = -0.89$, $p < 0.001$) (Fig. 7).

Discussion

Large scale climate patterns, in this study illustrated by NAO indices, were better predictors of the annual phytoplankton development in Pärnu Bay than local environmental variables such as riverine runoff (i.e. nutrient input), salinity and temperature. As a result of the shallowness of the sampling area, there exist a strong coupling between the severity of winters and the NAO values in Pärnu Bay. Besides temperature effects, higher values of the NAO indices were related to the better annual light conditions, indicating the prevalence of drier seasons after warmer winters in the study area. The NAO values, involving complex weather patterns e.g. temperature and wind effects (Ottersen et al., 2001), are likely to determine the timing and magnitude of phytoplankton spring peaks. Higher values of the NAO result in the warming of seawater and, hence, increase the length of the growing season of the phytoplankton (Reid et al., 1998). A lower amplitude of phytoplankton spring blooms at high NAO values in March is likely to be associated with higher frequency of stormy weather (i.e. high turbulence) and poorer light conditions. Besides the effects of large-scale climate patterns, the strong decline of phytoplankton biomass in the early 1990s is likely to be related to the lower loads of nutrients by better water treatment and lower intensity of agriculture in the catchment area.

For the higher trophic levels the direct effect of the NAO was not detectable; probably it was shaded by trophic interactions (cf. Ottersen et al., 2001). The results of this study demonstrate that zooplankton dynamics were poorly related to the NAO development but were a function of surface temperature and vertical temperature gradients instead. The annual cycle of the abundance dynamics of zooplankton in Pärnu Bay is known to be dependent on water temperature (Ojaveer et al., 1998; Simm & Ojaveer, 2000). Higher temperatures increase growth rates and enables the building up of larger zooplankton populations (Möllmann et al., 2000). Strong temperature gradients are often related to higher biological productivity in the area (Fournier, 1978; Kahru et al., 1984; Loder & Platt, 1985). This is also supported by the aggregation of zooplankton (Ojaveer & Kalejs, 1974; Ojaveer & Simm, 1975), mysids (Kotta & Kotta, 1999, 2001) and fish (Ojaveer & Kalejs, 1974; Ojaveer et al., 1998) towards the different frontal areas of the Gulf of Riga. Elevated densities of zooplankton refer to higher productivity of zooplankton food items i.e. protozoan (Ciliata) and phytoplankton on such fronts. However, weak correlations between phytoplankton and zooplankton densities suggest the significance of other factors.

The dynamics of zooplankton involves two major processes in Pärnu Bay: (1) climate induced variability and (2) biological invasions with concurrent changes in trophic interactions. Since the early-1990s two successful invaders have been observed in increasing numbers in Pärnu Bay. The predatory cladoceran *Cercopagis pengoi* was discovered in 1991 (Ojaveer & Lumberg, 1995; Ojaveer et al., 1999). Another invader is the spionid polychaete *Marenzelleria viridis* (Kotta & Kotta, 1998) whose larvae nowadays are the most prevalent taxon in meroplankton and has doubled the meroplankton abundance. *C. pengoi* is likely to have a stronger effect on the ecosystem of Pärnu Bay than *M. viridis* larvae. Prior to the invasion of *C. pengoi* the development of zooplankton (especially copepods) was significantly related to phytoplankton dynamics. After the invasion of *C. pengoi* the competitive and predator-prey interactions are likely to determine the outcome of zooplankton community.

We observed a link between the density of mysids and that of copepods and rotifers. This suggests that zooplankton forms the main diet of mysids in the study area (Rudstam et al., 1989; Hansson et al., 1990). Since 1991 these relationships are weaker and significantly deviating when the density of *C. pengoi* is high. As they are feeding on the same food items (e.g. Hansson et al., 1990; Rivier, 1998), a strong competition between *C. pengoi* and *N. integer* is likely. This is also indicated by the strong negative correlation between the density of *N. integer* and *C. pengoi*.

Considering the lack of significant correlations between the abundance of herring larvae and zooplankton stock in summer, it is likely that food

was not limiting the development of herring larvae before 1990s as it might be expected if clupeids exert a strong top-down control on mesozooplankton. However, Rannak & Simm (1979) demonstrated a significant positive correlation between herring year-class abundance and the availability of suitable food (copepod nauplii) for herring larvae during their transition to external feeding. This implies that the effect of zooplankton on herring breeding success is probably year-specific, depending on the temporal and spatial pattern of zooplankton and larval patches. Since the early 1990s fish predation did contribute to the seasonal development of the major zooplankton taxa, suggesting that clupeid stock size cascades down to the trophic level of mesozooplankton. Besides the changes induced by *C. pengoi*, the increasing effect of herring on zooplankton is likely to be amplified by a sharp increase in herring stock size in the Gulf of Riga since 1990 (ICES, 2001). Such top-down control of planktovorous fish on zooplankton has been reported earlier for the Baltic Sea proper (Hansson et al., 1990; Rudstam et al., 1994).

In conclusion, the results of this study indicate the prevalence of bottom up control of pelagic communities in Pärnu Bay before the 1990s. However, the higher trophic level interactions shaded the direct effects of the phytoplankton on mysid and fish communities. Since 1991, the development of pelagic communities are strongly affected by the invasion of the predatory *C. pengoi*. Strong predation pressure of *C. pengoi* on other zooplankton taxa has partly released phytoplankton from zooplankton grazing and resulted in food limitation of mysids and herring larvae.

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SOCIETY INFORMATION

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